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A STATISTICAL EVALUATION OF THE LEUCOPENIC INDEX IN ALLERGY*

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A CRITICAL investigation of the leucopenic index, which has received so much recent attention in the field of allergy, was begun by us early in 1936 and discussed in a preliminary report.† The present paper will report in full a series of experiments with this test as applied to both non-sensitive and food-sensitive individuals. Since it became apparent early in the investigation that the results of the leucopenic index test are dependent to an important degree upon the conditions and technique involved, the test has been carefully standardized and modified so that error would be minimized and controlled.

HISTORY OF THE LEUCOPENIC INDEX

As early as 1914, Widal, Lermoyer, Abrami, Brissaud, and Joltrain[1] described a "crise hemoclastique" which occurred before an asthma attack in a man exposed to sheep dander. This hemoclasia had previously been observed in anaphylactic animals and consisted of lowered arterial pressure, altered coagulability and refractometric index, and peripheral leucopenia. The leucopenia alone was found to be a satisfactory index to the condition. In 1920, Widal, Abrami and Iancovesco[2] reported that the portal blood of dogs produced hemoclasia if, following a protein meal, it was allowed to enter the dog's general circulation without first passing through the liver. Assuming that faulty liver function in man would permit a similar hemoclasia, Widal proposed the "digestive hemoclasia" test for liver dysfunction. It consisted of one fasting leucocyte count before a protein meal (usually 200 c.c. of milk), and postprandial counts at twenty and forty minutes. The test was called positive if postprandial leucopenia was found.

The hemoclastic test was used as a diagnostic aid not only in allergy and liver disease but in other medical conditions as well. Robertson,[3] for example, asserted that 94 per cent of her mental cases gave a positive test and also reviewed the work of others who had applied it to epilepsy and pregnancy.

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In time, however, controlled investigations discredited the test. Shaw,[4] for example, showed that the test measured only the normal fluctuation in successive leucocyte counts. Sabin, Cunningham, Doan, and Kindwall[5] reported large fluctuations in normal subjects. Twenty cases of proved liver disease tested by Goodman and Connery[6] showed similar variations in leucocyte level, regardless of whether milk or no food had been taken. Power and Pettit[7] found no evidence of digestive leucocytosis or leucopenia in their control and test studies upon 50 insane. Yates and Batt[8] observed no significant changes in the leucocyte count after their 34 mental cases ingested milk; and when they analyzed 24 previous reports upon the hemoclastic crisis, they found that most of the fluctuations fell within the limits of error surrounding cell counts.

In 1934, Vaughan[9, 10] revived Widal's "digestive hemoclasia" test, renamed it the "leucopenic index," and has since (Vaughan[11-13]) been advocating it as a diagnostic aid in food allergy. He claims that a correspondence of from 81 to 93 per cent exists between clinical food sensitivity and a postprandial leucopenia of 1000 or more cells. As in past periods, the digestive hemoclastic test of today has numerous supporters. Rinkel[14] has reported that clinical evidence of food allergy was obtained in 90 per cent of cases giving a positive leucopenic index, whereas compatible foods uniformly led to a leucocytosis. Rinkel and Gay[15] have highly recommended the method after using it 5000 times in various allergies, including habitual hyperthermia and peptic ulcer. Contending that peptic ulcer may be associated with food sensitivity, Gay[16] reported instances where ingestion of incompatible food led to a drop not only in the total leucocyte count but in gastric acidity as well. (Long[17] was unable to find any such correlation between leucocyte level and gastric acidity.) Zeller[18] found the index of decided value in his etiologic studies on intractable asthma, as did also Dean and his collaborators[19] in nasal allergy. Squier and Madison[20] increased the efficiency of their leucocyte studies by concurrent eosinophile determinations. Denny,[21] after doing over 4000 leucopenic index tests, described 6 cases to exemplify the diagnostic value of the method but unfortunately gave no data regarding the general efficiency of the test. The *Journal of the American Medical Association*[22] has suggested that adequate allowance for physiologic fluctuation and for error of method be made in utilizing the leucopenic index test.

I. TECHNIQUE OF LEUCOPENIC INDEX TEST AS PRESCRIBED BY VAUGHAN AND OTHERS

Although some of the early indices were obtained in the afternoon (after five hours' fast) by Vaughan[9] and by Rinkel,[14] most tests are now started in the early morning with the patient in the fasting state. Vaughan obtains the first fasting count upon the patient's arrival and the second count ten minutes later, and uses the average (or more recently the lower) of the two counts as the fasting level against which postprandial counts are plotted. Although, according to Sullivan,[23] Vaughan permits his patients to go out to a restaurant for their test food, Rinkel and Gay,[15] and Zeller[24] recommend seated rest throughout, and for thirty minutes or more prior to the test. Rinkel and Gay prohibit smoking and unnecessary conversation during the test period, and Zeller[18] warns that emotions may disturb the white count. Rinkel[14, 15] specifies that the test food shall have been included in the diet regularly prior to the test, that its preparation be proper, and that its

ingestion on the test day be completed within five minutes. A series of postprandial counts is obtained at fifteen-, twenty-, or thirty-minute intervals, depending upon the personal preference of the physician, over a period of an hour or more. A fall in any postprandial count of 1,000 or more cells from the fasting level is termed "positive" by any standard. Vaughan classifies a leucopenia of 500 to 1,000 cells as "borderline"; and any fall of less than 1,000 cells is considered "indeterminate" by Rinkel and Gay. Exception is made in the case of postprandial counts, which remain practically at the fasting level for twenty to forty minutes and then show a marked rise. This type No. 6 response is termed "positive" by Rinkel and Gay who find it almost invariably associated with incompatibility. Rinkel[25] holds that a leucopenia should be found preceding the leucocytosis providing frequent counts are made at five-minute intervals.

Early articles by Vaughan gave no details concerning technique, except that the same apparatus was used for all tests upon a given patient. Recently, he[13] specified that the blood sample be obtained from a freely flowing needle puncture, the pipette shaken three minutes, the counting chamber filled evenly, and at least 200 cells (8 sq. mm.) be counted for each determination. Rinkel and Gay modify this technique by shaking the pipette with a "simultaneous end-to-end movement." Denny[21] still further improves the technique by counting 16 sq. mm., but risks inaccuracy by shaking his pipette only sixty times. Rinkel, Gay, and Zeller claim 5 per cent as their maximum technical error; and Sullivan[23] stated that Vaughan judges his accuracy by the proximity of his two fasting counts, which differ from each other by 500 cells or less in 89 per cent of his cases.

II. REVISED TECHNIQUE USED IN PRESENT EXPERIMENT

A. Description of Technique.

Except where the response was known to be explosive, the test food was included in the daily diet of prospective test subjects up to within two days of the test. For twelve hours prior to the early morning test all food, alcoholic beverages, smoking, adrenalin, and strenuous exertion were interdicted. If emotional states became evident during the test period, results were discarded. At least thirty minutes of relaxed sitting were required prior to the onset of the test, which consisted of three fasting counts at twenty-minute intervals, ingestion of a test food during approximately a five-minute period, and three postprandial white counts obtained at twenty, forty, and seventy minutes. The only activity permitted during the entire test was a slow, short walk of about five yards to and from the laboratory for each blood count. Free bleeding usually occurred spontaneously if twenty to forty seconds were allowed to elapse after the stab, but venous compression at the base of the finger was sometimes required. Successive

fingers were used, and the first four large drops of blood were discarded. After a 1:20 dilution with 3 per cent acetic acid, the contents of the pipette were shaken in a slow, rotary motion, first described by Potain[26] and recently advocated by Bryan and Garrey,[27] for a full five minutes. Three minutes of settling were allowed after the counting chamber had been cautiously filled by capillarity, according to Osgood,[28] and approximately 800 cells (600 to 1,000), or 12 to 36 sq. mm., of counting surface were counted. Levy double counting chambers with improved Neubauer ruling, certified by the United States Bureau of Standards, were used in conjunction with Zeiss pipettes. Each subject had all his blood counts performed by one technician who used constant apparatus. The approximate total error of each technician using her assigned apparatus was determined in a manner to be described under "Experimental Data."

B. Sources of Error Controlled by Revised Technique.

The above modifications in technique are justified not only by our own experience with the leucopenic index but by the literature on the white cell count, which shows that important fluctuations may occur due to (1) numerous physiologic and psychic states in the subject and to (2) errors of method.

1. *Physiologic Factors.*—a. Afternoon rise. All counts were begun in the early morning in order to obviate the possible influence of the so-called "afternoon rise," with its increased fluctuations as described by Mauriac and Cabouat,[29] Shaw,[30] Sabin,[5] Garrey and Butler,[31] Zirm and Bauermeister[32] and others, and found inconstantly by Bryan, Chastain, and Garrey[33] and by Ponder, Saslow, and Schweizer.[34]

b. Activity. It is important that adequate rest be given the subject prior to starting the test. The literature is replete with evidence of the leucocytosis which results from exertion. That random activity, such as laboratory work, increases the leucocyte level has been denied by Shaw,[30] Schweizer,[35] Jones, Stephens, Todd, and Lawrence.[36] However, Garrey and Butler[37] and also Bryan, Chastain, and Garrey[38] concluded that mild activity may be associated with slight increase in the white count. In our early tests, it was our practice to commence the blood determinations shortly after the patient arrived, but so definite was the trend for the initial count to be high that we were forced to discard it and institute a half hour of preliminary rest in subsequent counts. Study of the individual blood counts (listed completely in Table VI) revealed that a high proportion (85 per cent) of our rested, normal adults showed a fasting average comparable to the "low basal level" which Bryan, Chastain, and Garrey[38] found in their recumbent group after an hour of rest. Of their 200 subjects, 95 per cent had counts below 7,800 cells. Fasting levels as high as 10,500 and 16,500 cells (reported in some of Vaughan's patients by Sullivan[23]) suggest physiologic unrest, emotional influence or technical

inaccuracy. Such factors may account for the day to day variability in fasting level found by Vaughan[9] in his first reported patient, whose fasting count was as low as about 7,500 cells on one day and as high as to 12,500 cells on another.

c. Physiological oscillation. Rinkel and Gay,[15] and Zeller[18] depend upon one fasting count, despite the fact that oscillation is universally accepted as characteristic of the normal white count. Ponder,[34] for example, reported that normal subjects during mild activity show "residual" fluctuations of as much as ±8 per cent throughout the day, "even after all errors of method have been allowed for." Similar fluctuability was found in our 19 normal individuals, on whom 3 fasting counts were obtained in accordance with the modified technique (Table VI). The fasting range observed in these subjects averaged roughly 1,100 cells, the fasting level roughly 7,000 cells, and the standard deviation* from the fasting mean of the group was estimated as ±517 cells, as Table III indicates. (±8 per cent of a white count of 7,000 cells would amount to ±560 cells. Hence, the fluctuation in our normal group approximated that reported by Ponder.) Inspection of the fasting counts obtained upon our Group I normal subjects (Table VI) will reveal that over one-third (8 out of 19) of these subjects showed a fasting range of 1,000 cells or more in some test, and of these one subject had a range of 2,000 cells. Had the test food, by chance, been given between the two extreme counts in such instances, only one fasting count being obtained, the resulting "positive leucopenic index" would have represented merely a normal fluctuation of the individual rather than food incompatibility.

Zeller,[24] after finding small fasting ranges not exceeding 750 cells in his seven seated subjects, believes that larger fluctuations arise from extraneous sources, such as activity. Aside from the questionable influence of short walks to and from the laboratory, our subjects were carefully guarded from all extraneous sources of variation. However, even with the improved technique, only 18 of the 39 tests on normal subjects showed a fasting range of 500 cells or less; 7 had a range

*The fluctuation of successive white counts about their mean value is best measured by the standard deviation. The standard deviation is usually defined as the square root of the arithmetic mean of the squares of the deviations of the various items from the arithmetic mean of the whole. Modern statistical methods may, however, be applied more conveniently if the sum of squares is divided by a number less by unity than the sample number. Symbolically, where m stands for the mean of the observation, Σ for summation, x for an individual observation, and n for the number of observations, we define the arithmetic mean as

$$m = \frac{\Sigma x}{n},$$

and the standard deviation as

$$\sigma = \sqrt{\frac{(x - m)^2}{n - 1}}.$$

The standard deviation of the mean of a number of observations, n, is equal to the standard deviation divided by the square root of n, $\sigma/\sqrt{n}$. The meaning of this statement is that, if σ is the standard deviation of the individuals in a population of N individuals, and if we form all possible groups of n in this population, then the standard deviation among the means of these groups approaches $\sigma/\sqrt{n}$ as N increases.

between 501 and 700 cells; 4 a range between 701 and 999; 9 a range between 1,000 and 1,999 cells; and one had a range of 2,000 cells. Reference to Table I will show that smaller oscillations were *at times* present in 3 members of our normal group who were kept fasting and strictly inactive over the entire test period. That stability varies from person to person and from day to day, as mentioned in the literature (Doan and Zerfas,[39] Smith and McDowell,[40] Shaw,[41] Stetson,[42] and Bryan and his collaborators[38]), is demonstrated by this experiment. Table I will make obvious the possibility of obtaining low fasting ranges in groups as small as Zeller's, but in a larger series of cases, greater ranges must inevitably be found despite the most refined technique. One cause for this is the inescapable error due to unequal distribution of cells on the counting chamber, which will be discussed under "technical errors." In our opinion, a reliable fasting level should be based on not less than three blood counts.

TABLE I

SERIAL LEUCOCYTE COUNTS OBTAINED ON THREE NORMAL SUBJECTS KEPT FASTING AND STRICTLY INACTIVE (MEMBERS OF GROUP I)

DATE	SUBJECT	POSTURE	NO. OF COUNTS AT 20-MINUTE INTERVALS	MEAN LEUCOCYTE LEVEL	OBSERVED TOTAL RANGE	STANDARD DEVIATION	COEFF. OF VARIATION*
12/7	Male Aged 8	sitting	10	9762	1362 cells	±470	4.8 per cent
12/8	Male Aged 8	sitting	10	9244	1400 cells	±470	5.1 per cent
12/9	Male Aged 8	sitting	5	7744	480 cells	±192	2.5 per cent
11/4	Female Aged 7	recumbent	8	6056	1006 cells	±351	5.8 per cent
11/5	Female Aged 7	recumbent	8	6360	316 cells	±113	1.8 per cent
11/9	Female Aged 7	recumbent	9	6424	375 cells	±103	1.6 per cent
11/10	Female Aged 7	recumbent	10	6443	609 cells	±190	2.9 per cent
11/11	Female Aged 7	recumbent	8	6319	344 cells	±105	2.1 per cent
11/13	Female Aged 7	recumbent	7	6385	217 cells	± 85	1.3 per cent
11/26	Male Aged 28	recumbent	8	6536	523 cells	±183	2.8 per cent

*Standard deviation expressed as per cent of the mean leucocyte count.

The table shows that the leucocyte oscillation and average differed somewhat from person to person and from day to day, even though activity was not a factor.

d. Emotions, drugs, vomiting. Probably through their effect upon the vasomotor system, which influences the distribution of white cells, such drugs as alcohol (Medlar[43]) and adrenalin (Fegler[44]) alter the peripheral white count; as do also nausea, vomiting, mental strain, and a variety of emotions, such as pain and apprehension (Garrey and Butler[31]).

2. *Errors of Method.*—In addition to the physiologic fluctuation in the leucocyte count, there are three important sources of error in the technical procedure of blood counting.

a. Failure to procure representative sample. Shaw[45] showed that peripheral blood counts were representative of systemic blood, providing local vasoconstriction was obviated. He obtained peripheral vasodilatation and representative counts by means of local friction or external warmth. Bryan, Chastain, and Garrey[46] accomplished the same by merely awaiting the reflex vasodilatation which follows the lancet stab by twenty to forty seconds and then discarding the first four drops of blood before withdrawing the sample. Local vasoconstriction causes definite increase in the white count and thus may introduce large error.

b. Inadequate mixing of cells and diluent. Bryan and Garrey[27] have shown that blood counts, based upon the customary shaking of the pipette in one plane, have a range of variation which is four times greater than that of counts based upon Potain's[26] principle of rotary mixing. Our counts are based upon manual rotation of five minutes' duration.

c. Failure to count an adequate number of cells. When all other errors in the leucocyte count have been minimized, there remains the large and inescapable error of random sampling due to the chance distribution of cells on the ruled field of the counting chamber. Student[47] has shown that the distribution of cells on the surface of the hemocytometer follows the Poisson law, the error of random sampling diminishing as the number of cells counted is increased. Yates and Batt,[8] in studying the hemoclastic crisis test, calculated the standard error or standard deviation of random sampling by obtaining, in accordance with the Poisson law, the square root of the number of cells enumerated, counts of 100 cells thus having a standard error of $\pm$ 10 cells, or a coefficient of variation of $\pm$ 10 per cent; counts of 200 cells, a coefficient of variation of $\pm$ 7 per cent; counts of 400 cells, a coefficient of $\pm$ 3.5 per cent. No matter how faultless the technique, an error of the magnitude indicated in this Poisson series may occur in enumerating, because of unequal cell distribution. When Ponder and his associates[34] determined the relative errors in cell counts of 200, 400, and 800 cells, the errors they observed approximated the corresponding standard error as estimated above. Both statistical theory and experimental evidence, therefore, lead one to conclude that the error of distribution involved in any 8 sq. mm. leucocyte count may at times amount to more than the 5 per cent maximum error claimed by Rinkel, Gay, and Zeller. At a level of 7,000 cells, 8 sq. mm. would contain less than 300 cells, with a standard error of $\pm$ 5.6 per cent.

At a level of 7,000 cells, this would amount to 392 cells upward or downward fluctuation, or a total range of 784 cells, which might be expected to appear at times because of faulty cell distribution. Hence, Zeller's small fasting ranges of 750 cells or less would be exceeded by the distribution error of 8 sq. mm. counts, and larger fasting ranges would be expected to appear, providing a sufficient number of cases were studied. Denny[21] has definitely improved the leucopenic index technique by enumerating 16 sq. mm. for each leucocyte determination, thus reducing the coefficient of variation of a blood count at the level of 7,000 cells to ± 4 per cent by counting 600 cells. However, some other inaccuracy may have been introduced into his work, because only one of his 32 charted indices showed a fluctuation of less than 1,000 cells from the fasting level, whereas 46 of our 60 indices for comparable patients varied less than 1,000 cells up or down from the fasting average. Denny's dependence upon one fasting count and his method of shaking the pipette may account for his wider fluctuations.

For a detailed and comprehensive review of the causes of variations in white blood cell counts, the reader is referred to the paper by Garrey and Bryan.[33]

EXPERIMENTAL DATA

I. PRELIMINARY ESTIMATION OF LIMIT OF ACCURACY IN COUNTING

In accordance with the method used by Ponder,[34] our three technicians simultaneously made consecutive counts upon a single sample of oxalated blood in order to estimate the coefficient of variation (standard deviation expressed as percentage of the mean) for each technician using her constant apparatus. Using a blood sample containing 5,451 white cells per cubic millimeter and enumerating the cells (approximately 500) on 18 sq. mm. of surface for each determination, five such counts revealed a coefficient of variation of ± 2.8 per cent for one technician, ± 2.3 per cent for the second, and ± 4.7 per cent for the third. Ponder reported coefficients of variation of ± 3.3 per cent and ± 3.8 per cent, respectively, for his two technicians under similar circumstances. Inasmuch as none of these figures much exceeds the standard error calculated for 500 cell counts (± 4.4 per cent), the apparatus and technique of counting may be considered satisfactory.

II. EXPERIMENTS WITH LEUCOPENIC INDEX TEST

A. SELECTION OF SUBJECTS AND EXCITANTS; GROUPING OF RESULTS

Using the modified technique previously described, 121* leucopenic index tests were performed upon five groups of subjects. Group I

*Ten additional tests were done upon three members of Group I kept in the fasting state as shown in Table I.

consisted of 19 healthy adults with no past or present indications of allergy in themselves or their relatives. Groups II, III, and IV contained 29 patients, each of whom had clinical allergy to one or more foods. The six ragweed hay fever patients comprising Group V had on previous occasions given "constitutional" reactions to the subcutaneous injection of ragweed extract.

The test food selected for non-sensitive subjects was a standard breakfast of wheat, milk, and eggs. The members of Groups II, III, and IV were tested to incompatible food and, in as many instances as possible, also to compatible food. A suspected food was judged as incompatible only after its capacity to induce obvious allergic manifestations of *objective* nature in the patient had been repeatedly demonstrated. To be classed as compatible, a food must have been in the daily diet in large amounts for a long period of time without producing allergic symptoms. To aid the diagnosis, intradermal skin tests were done and, in many instances, the serum was tested on normal persons in passive transfer sites. Experiments with the ragweed-sensitive group differed from those of the food-sensitive groups only in that a subcutaneous injection of ragweed extract was substituted for the incompatible food, and one of normal saline for the compatible food.

Each set of 6 blood counts done in conjunction with incompatible food has been allotted to one of 3 groups, according to the patient's responsiveness, as can be seen in the first four columns of Table II. Some patients appear in two groups because of different responsiveness to different test foods, or to variable amounts of the same test food on different days. Group II contains the counts of those who, for some reason, *failed to react* in any observable way to the incompatible food on the occasion of the test. Group III contains the counts of patients whose major response was *delayed* until after the test had been completed. Since, with two minor exceptions, no allergic reaction was detected in this group until some time after the last blood count, we may assume that their white counts were not influenced by physiologic disturbances connected with allergic manifestations. The only evidences of allergy which were present during the test period in Group III appeared in two patients, one of whom developed transient itching of the mouth, and the other a small hive prior to the last blood count. Group IV, on the contrary, contains only those patients whose incompatible food caused *prompt* physiologic disturbances of a more or less violent nature. The influence of nausea, vomiting, pain, etc., upon the peripheral leucocyte count has previously been discussed and, as will be shown later, the occurrence of such symptoms during the seventy minutes postprandial period probably influenced the results in Group IV. Although suitable subjects were scarce and disinclined toward experimentation, various degrees and types of food idiosyncrasy are

TABLE II

MEAN DIFFERENCE BETWEEN FASTING AVERAGE AND LEUCOCYTE COUNTS OBTAINED 20, 40, AND 70 MINUTES AFTER INGESTION

GROUP	TOTAL NUMBER OF INDICES	TEST FOOD	SYMPTOMS ASSOCIATED WITH TEST	FASTING LEVEL OF GROUP	MEAN CHANGE FROM FASTING LEVEL AT 20 MIN. POSTPRANDIAL	STANDARD DEVIATION OF MEAN CHANGE	MEAN CHANGE FROM FASTING LEVEL AT 40 MIN. POSTPRANDIAL	STANDARD DEVIATION OF MEAN CHANGE	MEAN CHANGE FROM FASTING LEVEL AT 70 MIN. POSTPRANDIAL	STANDARD DEVIATION OF MEAN CHANGE	MEAN CHANGE OF 3 POSTPRANDIAL COUNTS FROM FASTING AVERAGE	STANDARD DEVIATION OF MEAN CHANGE
I Normal	39	Milk, egg, and wheat	None	6985	+ 69 cells	±120	+ 31 cells	±100	+178 cells	±105	+ 93 cells	± 87
II	3	Compatible	None	5204	-121 cells	±446	-413 cells	±346	-514 cells	±254	-349 cells	±329
Food-sensitive	13	Incompatible	None	5391	- 14 cells	±187	- 61 cells	±143	+160 cells	±186	+ 29 cells	±125
III	12	Compatible	None	5907	+ 38 cells	± 93	- 49 cells	±177	+ 62 cells	±236	+ 17 cells	±137
Food-sensitive	15	Incompatible	Delayed	6787	- 56 cells	±140	-153 cells	±183	- 53 cells	±198	- 89 cells	±133
IV	8	Compatible	None	6422	-251 cells	±183	- 67 cells	±134	+ 49 cells	±165	- 97 cells	±120
Food-sensitive	9	Incompatible	Prompt; acute	6345	-254 cells	±338	+116 cells	±316	+175 cells	±275	+ 12 cells	±226

When each of the above changes from the fasting average was compared with its standard deviation, reference to Student's "t" table revealed that in no instance was the mean change sufficiently greater than its standard deviation to be of statistical significance. Ingestion, therefore, was not found to influence significantly the average leucocyte level of any group within the seventy-minute postprandial period.

represented in these allergic groups and should serve as a satisfactory basis for estimating the relationship between incompatibility and the leucopenic index.

B. A STATISTICAL ANALYSIS OF THE PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS OF THE VARIOUS GROUPS

This will be presented under two headings. The first will analyze the blood counts as prescribed by the advocates of the leucopenic index; that is, a comparison will be made between the *leucocyte level* at each of the three postprandial periods and the fasting average (based on three fasting counts in most of our cases). Since this method does not adequately take into account the *leucocyte oscillation*, under a separate heading we have determined the relative fluctuation of the fasting period and compared this with the corresponding postprandial period. In general, data have been considered in groups, since this simplifies final comparisons and minimizes the influence of individual peculiarities in the leucocytic response.

1. *Comparison of Postprandial Leucocyte Levels With Fasting Average.*—Vaughan[12, 13] states that there is "a definite tendency toward a lower range of the digestive leucocyte response" after an incompatible food has been ingested, and that the degree of depression in the leucocyte level corresponds quite closely with the severity of the clinical reaction. On the other hand, advocates of the leucopenic index test rely upon a "digestive leucocytosis" as a normal postprandial response.

In order to learn whether our normal subjects exhibited leucocytosis in any of the three counts after ingestion, the deviation of each postprandial count from its corresponding fasting average was calculated for each subject. All the deviations of counts obtained for the group at a given time, say twenty minutes after ingestion, were then averaged, as shown in Table II under the caption, "mean change from fasting average." It was thus found that a 69 cell rise was the mean change of Group I at the twenty-minute postprandial count. In order to determine whether this mean deviation could be attributed merely to random factors, it was necessary to compute its standard deviation. This was done by squaring the figure by which each blood count taken at the twenty-minute interval differed from the group average deviation, then dividing the sum of these squares by the number of observations less one. The square root of this figure is the standard deviation of the group, and from it the standard deviation of the mean change is calculated by dividing by the square root of the number of observations made on the group. In the example under consideration, the standard deviation of the mean change for Group I at the twenty-

minute interval was ± 120; and since 69 is less than 2.64* times this figure, the mean change is of no statistical significance.

Application of this treatment to the mean change of the normal group at forty and seventy minutes after ingestion of the standard breakfast revealed that in each instance the mean change was a small enough multiple of its standard deviation to be attributable to chance alone. As might be anticipated, the average obtained by pooling the three postprandial figures of the group was not significantly different from the corresponding average of all its fasting counts. (See the last two columns of Table II.) Hence, the experiment lends no support to the contention that ingestion is normally associated with leucocytosis, inasmuch as ingestion in our Group I was followed by no significant change in the leucocyte level throughout the seventy minutes the group was observed.

When similar statistical procedure was applied to the food-sensitive Groups II, III, and IV, no significant change from the fasting level was found at any postprandial interval in any group, whether compatible or incompatible food had been eaten (see Table II). The general postprandial average was likewise unchanged. The allergic groups resemble, therefore, the normal subjects in that compatible food failed to induce in them a postprandial leucocytosis. Their white counts after incompatible food were also statistically indistinguishable from the fasting average, thus lending no support to the contention that incompatibility is associated with leucopenia.

2. *Oscillation of the Leucocyte Count Before and After Ingestion.*—The fluctuation present in a series of leucocyte counts obtained on an individual is best measured by the standard deviation of those counts from their mean level. This is calculated as follows: The amount by which each count differs from the mean level is squared, added to the squares similarly obtained for the other counts, and their sum then divided by the number of observations less one. The square root of the result is the standard deviation as estimated from the counts upon the individual. If the relative stability of different *groups* is to be studied, a collective estimate of the group standard deviation must be made. The amount by which each count of a series on an individual differs from the mean of these counts is squared. All such squares obtained for a given group are then summed. This sum is divided by the number of degrees of freedom, and the square root of the quotient obtained.

*The figure 2.64 was obtained by entering Student's "t" table for the degrees of freedom on which the mean under consideration was based. The number of degrees of freedom is equal to the number of observations minus the number of means. Student's "t" table is used to determine the multiple of the standard deviation, as calculated above, which gives the range within which any desired percentage of the cases will fall, if random factors alone are operative. We have consistently used the figure corresponding to a probability of 1 in 100. Any change which fell outside this selected range was considered as probably being due to some factor other than chance; i.e., statistically significant. On the other hand, if the observation fell within this range, it was considered as being due to chance rather than any other factor.

This represents the standard deviation of the group. Any two such group standard deviations may then be compared, and the significance of their difference from each other determined through the application of Snedecor's modification of Fisher's "z" test,[48, 49] which will be illustrated in the next paragraph.

TABLE III

OSCILLATION OF LEUCOCYTE COUNT BEFORE AND AFTER INGESTION

GROUP	TEST FOOD	SYMPTOMS ASSOCIATED WITH TEST	STANDARD DEVIATION OF 3 PREPRANDIAL COUNTS*		STANDARD DEVIATION OF 3 POSTPRANDIAL COUNTS		SIGNIFICANCE OF DIFFERENCE BETWEEN S.D.† OF PREPRANDIAL AND POSTPRANDIAL COUNTS
			CELLS	DEGREES OF FREEDOM	CELLS	DEGREES OF FREEDOM	
I Normal	Egg, milk, and wheat	None	±517	76	±504	78	None
II Food-sensitive	Compatible	None	±324	6	±317	6	None
	Incompatible	None	±431	24	±524	26	None
III Food-sensitive	Compatible	None	±323	23	±468	24	None
	Incompatible	Delayed	±544	22	±525	30	None
IV Food-sensitive	Compatible	None	±441	14	±380	16	None
	Incompatible	Prompt Acute	±495	16	±777	18	Doubtful

*In a few instances, the initial fasting count was discarded from these calculations because of insufficient rest prior to the test. In such cases, two fasting counts were used.

†Standard deviation.

Table III shows the results obtained in Groups I to IV, inclusive, when comparisons were made in this manner upon the counts obtained in each group before and after ingestion. For example, the standard deviation calculated for Group I was ±517 cells in the case of the preprandial counts and ±504 cells in the postprandial counts. Obviously, if the ratio between the preprandial and postprandial standard deviation were 1, no change in the oscillation would have existed. In Group I the ratio of the squares of the standard deviation is not 1, but the difference from 1 was found to have no statistical significance when Snedecor's table* was consulted. When similar comparison between the preprandial and postprandial fluctuation in each of the remaining groups was made, a statistically significant value of F was found only in Group IV, where the degree of oscillation shown after the ingestion of incompatible food was sufficiently increased over the preprandial fluctuation to be of possible significance. (The probability that so great a difference should arise by chance alone was found to be slightly less than 1 to 20.) It will be noted in Table VI that 9 of the 12 tests in this group were complicated by the prompt development of nausea, vomiting, or abdominal colic, all of which are known to disturb the leucocyte count.

*Snedecor has adopted the symbol F to represent the ratio of the square of the larger standard deviation to the square of the smaller standard deviation, and his table gives values which F will equal or exceed only once in a hundred trials due to chance alone, when entered for the correct degrees of freedom.

Following the above procedure, the standard deviation listed in Table III for each of the allergic groups was compared with the corresponding preprandial or postprandial figure for the normal group. This treatment of the preprandial period revealed that no group had a significantly different standard deviation from that of the normal group except Group III. We are unable to explain why a definitely significant diminution in fluctuation appeared in this group prior to the ingestion of compatible food, but the increase in stability was apparent when comparison was made with the preprandial fluctuation not only of Group I but also of Group IV prior to the latter's tests to incompatible food. Inasmuch as this same Group III failed to exhibit diminished fluctuation in its fasting counts on the days incompatible food was to be tested, its anomalous stability prior to compatible food should not be given too much weight.

Similar comparisons for the postprandial period revealed that the allergic groups resembled Group I in all instances except Group IV, whose incompatible foods were followed by increased leucocyte fluctuation of definite significance.

C. INCIDENCE OF POSTPRANDIAL LEUCOPENIA IN INDIVIDUAL SUBJECTS

Having ascertained the standard deviation of our normal group, it is now possible to determine what constitutes roughly a significant drop in the individual leucocyte counts of our test subjects. This may be calculated as follows: Inasmuch as the standard deviations found in the normal group before and after ingestion are statistically similar, they may be weighted and pooled, giving ±511 cells as the resulting standard deviation for the one-hundred-and-fifteen-minute period. (This figure may be converted into the coefficient of variation by dividing it by 7,000, the approximate level of all the leucocyte counts done on Group I, and multiplying the result by 100. The answer is ±7.3 per cent.) Multiplying the standard deviation, ±511 cells, by the factor 2.58,* one obtains ±1,318 cells. Classifying each of the three postprandial counts of members of the normal and allergic groups on this basis, we find that 3 of the 117 counts of Group I showed a fall of 1,318 cells or more from the corresponding fasting average, whereas this degree of fall is found in 2 of the 111 counts upon allergic individuals following incompatible food, and in 2 of the 69 counts upon allergic individuals following compatible food. Because of the small sampling or some other factor, the observed incidence was somewhat higher than the theoretical 1 in 100, but the important fact to note is that

*The range within which 99 per cent of the individual observations in a normally distributed population will fall is known to be 2 by 2.58 (or 5.16) by σ. Applying this factor to our Group I standard deviation of ± 511 cells, we obtain 2637 cells as the range (1318 cells up or down from the mean level) within which 99 per cent of the counts upon a large population analogous to our Group I would fall if no other causes of fluctuation were introduced except chance.

postprandial leucopenia of a given degree was present as often in normal subjects and in allergic individuals following compatible food as it was in allergic cases following incompatible food. No impressive difference was found between normal and allergic cases when we borrowed Vaughan's criterion and determined the incidence of a leucopenia of 1,000 or more cells in any one of the three postprandial counts of our subjects. Drops of this degree were found in 4 of the 37 subjects tested to incompatible food, in 2 of the 23 food-sensitive subjects tested to compatible food, and in 3 of the 39 normal persons. In our experiments, therefore, leucopenia was no more characteristic of the postprandial period of allergic individuals given incompatible food than of normal or allergic individuals given compatible food.

The failure of incompatible food to cause postprandial leucopenia is very well illustrated by the experiments upon a patient, H. B., whose

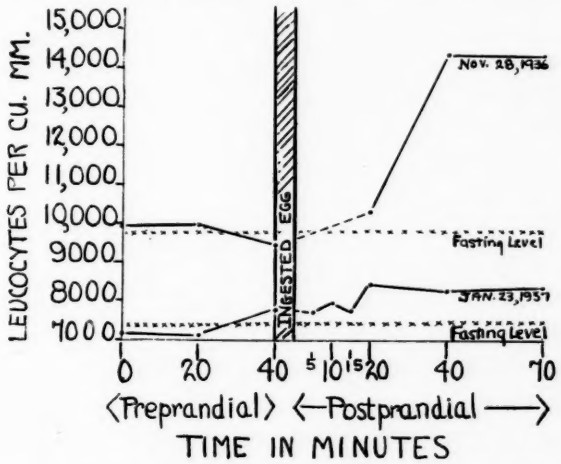

Fig. 1.—Duplicate leucopenic index tests on Case H. B., highly sensitive to egg. No leucopenia was found after ingestion even when counts were made at five-minute intervals.

tests are recorded in Table VI, even though they were excluded from all calculations because of the patient's emotional instability. (The influence of emotion upon her leucocyte count probably accounts for the instability apparent in her preprandial counts listed in Group II.) In Group IV, this patient was tested three times to raw egg, which invariably led to prompt nausea, vomiting, and abdominal pain, complicated by asthma in one test and symptoms of hay fever in another. In no test, however, was significant leucopenia found after ingestion, even though the test was repeated in order that blood samples might be taken every five minutes during the early postprandial period (see Fig. 1). The latter procedure was advised by Rinkel,[25] who contends that curves like the upper one in Fig. 1, resembling his[15] "curve No. 6," probably would show an initial leucopenia if frequent samples were obtained prior to the definite leucocytosis.

D. LEUCOCYTIC RESPONSE TO INJECTIONS OF POLLEN EXTRACT

Since ingestion exerted no significant effect upon the postprandial leucocyte behavior of any group except Group IV, whose incompatible food induced prompt and acute physical disturbances, an attempt was made to investigate the effect upon the leucocyte count of an allergic reaction induced by pollen extract. Accordingly, leucopenic index tests were performed upon six ragweed-hay fever patients (Group V) who abstained from food and in its stead received subcutaneously a dosage of ragweed extract which had previously induced a "constitutional reaction." Table VI indicates the type of reaction which occurred in these patients during the seventy-minute period following injection. Reactions were slight or moderate in degree. In each case, the test was repeated under identical circumstances except that saline solution was injected instead of pollen extract.

As far as leucocyte *level* is concerned, Table IV reveals that in no one of the three counts taken after injection of ragweed extract or saline solution did the group average differ significantly from its preinjection level. As would be expected, the average change of all postinjection counts from the mean preinjection level of the group was insignificant.

TABLE IV

MEAN DIFFERENCE BETWEEN PREINJECTION AVERAGE AND LEUCOCYTE LEVEL 20, 40, AND 70 MINUTES AFTER INJECTION

(Group of Six Ragweed-Sensitive Cases)

AFTER INJECTION OF RAGWEED EXTRACT	MEAN CHANGE FROM PREINJECTION LEVEL	STANDARD DEVIATION OF MEAN CHANGE
20 minutes	+232 cells	±270 cells
40 minutes	+163 cells	±287 cells
70 minutes	- 5 cells	±287 cells
Average	-130 cells	±212 cells
AFTER INJECTION OF SALINE SOLUTION		
20 minutes	+ 37 cells	±170 cells
40 minutes	+369 cells	±293 cells
70 minutes	+ 35 cells	±275 cells
Average	+147 cells	±185 cells

TABLE V

OSCILLATION IN LEUCOCYTE COUNTS TAKEN BEFORE AND AFTER INJECTION OF RAGWEED EXTRACT AND OF SALINE SOLUTION

(Group of Six Ragweed-Sensitive Cases)

INJECTION ADMINISTERED	STANDARD DEVIATION OF 3 COUNTS BEFORE INJECTION	STANDARD DEVIATION OF 3 COUNTS AFTER INJECTION	SIGNIFICANCE OF DIFFERENCE BETWEEN PRE- AND POSTINJECTION STANDARD DEVIATION
Ragweed extract	±525 cells	±410 cells	None
Saline solution	±460 cells	±507 cells	None

TABLE VI

GROUP I

NON-SENSITIVE CONTROL GROUP

NAME	PREPRANDIAL WHITE COUNT			TEST FOOD	POSTPRANDIAL WHITE COUNT			SYMPTOMS INDUCED BY TEST
	1ST	20 MIN.	40 MIN.		20 MIN.	40 MIN.	70 MIN.	
E. P.	7037*	6900	6525	Milk, egg, and wheat	6363	6525	6887	None
F. H.	8100	7500	7650	Milk, egg, and wheat	7350	6460	8000	None
	7290	7380	7440	Milk, egg, and wheat	7150	6970	7010	None
	7580	7670	6530	Milk, egg, and wheat	7790	8960	9030	None
	6439	6630	6589	Milk, egg, and wheat	6199	6664	6722	None
	6461	6522	6639	Milk, egg, and wheat	6364	6681	6581	None
O. H.	8487	7650	7030	Milk, egg, and wheat	7510	8270	8887	None
	10513	7600	7870	Milk, egg, and wheat	7060	7850	7060	None
	7400	6460	7150	Milk, egg, and wheat	6890	7060	8240	None
	6810	6780	7360	Milk, egg, and wheat	7300	6340	7220	None
	7320	6820	7180	Milk, egg, and wheat	7350	6900	6760	None
	6322	6610	6570	Milk, egg, and wheat	7010	6840	6456	None
F. K.	7070	6970	7050	Milk, egg, and wheat	6770	7030	7110	None
	7820	7410	7896	Milk, egg, and wheat	7200	6880	7160	None
	6697	6289	6489	Milk, egg, and wheat	7122	6223	6156	None
	6197	6625	6247	Milk, egg, and wheat	6781	5973	6139	None
	6364	6397	6372	Milk, egg, and wheat	6897	6189	6341	None
	6564	6397	6511	Milk, egg, and wheat	6955	6416	6514	None
	6414	6502	5859	Milk, egg, and wheat	6739	6339	6297	None
	6422	6472	6297	Milk, egg, and wheat	6677	6456	6331	None
S. W.	9413	9637	8580	Milk, egg, and wheat	8660	8510	8520	None
	11887	10513	11687	Milk, egg, and wheat	11275	10700	12313	None
	9539	9637	10313	Milk, egg, and wheat	10813	10100	11425	None
	12750	12075	10750	Milk, egg, and wheat	11750	12763	12113	None
	9137	8290	8088	Milk, egg, and wheat	10413	10100	9275	None

*This initial count was excluded from calculations because of inadequate rest prior to onset of test.

GROUP I—CONT'D

NAME	PREPRANDIAL WHITE COUNT			TEST FOOD	POSTPRANDIAL WHITE COUNT			SYMPTOMS INDUCED BY TEST
	1ST	20 MIN.	40 MIN.		20 MIN.	40 MIN.	70 MIN.	
M. C.	9513	8950	9113	Milk, egg, and wheat	6563	8724	8525	None
J. H.	4340	4273	4101	Milk, egg, and wheat	4900	4495	4840	None
M. L.	5614	5062	5200	Milk, egg, and wheat	4595	4755	4962	None
J. L.	8190	8010	8470	Milk, egg, and wheat	9460	9030	8350	None
A. D.	7399	9000	7410	Milk, egg, and wheat	8190	8211	7820	None
S. L.	5574	5588	4169	Milk, egg, and wheat	5448	5395	5454	None
Y. Z.	7232	8010	7580	Milk, egg, and wheat	8400	7890	6990	None
Y. Z.	6651	7222	5918	Milk, egg, and wheat	6786	6947	7022	None
I. G.	5574	5488	5368	Milk, egg, and wheat	4922	4982	6300	None
W. W.	3630	3330	3490	Milk, egg, and wheat	3857	3624	4273	None
D. A.	3485	4202	4842	Milk, egg, and wheat	4302	3546	5067	None
H. B.	5400	4129	4634	Milk, egg, and wheat	4788	4673	5245	None
C. M.	4046	3963	4201	Milk, egg, and wheat	4673	4129	3846	None
X. T.	6427	5867	6147	Milk, egg, and wheat	5967	6627	7053	None

GROUP II

ALLERGIC CASES WITHOUT SYMPTOMS ON OCCASION OF TEST

G. T.	4935	4656	4777	Incompatible	4240	4695	4928	None
	5716	5544	5467	Compatible	4612	4709	4856	None
J. S.	4868	4868	4928	Incompatible	5767	5501	5022	None
	5095	4575	5488	Compatible	5101	4415	4240	None
C. K.	5501	4962	5075	Incompatible	6227	5038	5421	None
R. R.	5794	4212	4529	Incompatible	5514	4895	4712	None
H. B.	10750†	7750	8780	Incompatible	9375	9925	10375	None
	10100†	9475	7450	Compatible	10125	9812	10787	None

†Entire test omitted from all calculations because of the emotional instability of the patient.

GROUP II—CONT'D

NAME	PREPRANDIAL WHITE COUNT			TEST FOOD	POSTPRANDIAL WHITE COUNT			SYMPTOMS INDUCED BY TEST
	1ST	20 MIN.	40 MIN.		20 MIN.	40 MIN.	70 MIN.	
R. H.	4800	5087	4787	Incompatible	4650	5437	4950	None
	5275	4962	4712	Compatible	5837	5250	4975	None
	6962	6437	6137	Incompatible	5625	5562	5837	None
	6375	5725	5690	Incompatible	5175	5262	5500	None
	5400	4725	4913	Incompatible	5363	5000	4813	None
	5600	4712	5250	Incompatible	5312	5525	5912	None
A. S.	9575*	7400	7700	Incompatible	7125	8250	9725	None
I. E.	5880	6240	6890	Incompatible	6680	6027	6154	None
Y. M.	7587	8762	7500	Incompatible	7350	7262	8212	None
P. H.	7305	7405	7355	Incompatible	7120	7172	7157	None

GROUP III

ALLERGIC CASES WITH SYMPTOMS AFTER THE 70-MINUTE COUNT

C. F.	7863*	6400	6513	Incompatible	6225	6500	6350	Headache, edema
	6538	6287	6475	Compatible	6188	6650	5975	None
R. H.	7030	7120	7060	Incompatible	6250	6740	6890	Dermatitis
	6670*	6890	6710	Compatible	6260	6710	6630	None
	6380	6530	6630	Incompatible	6240	6600	6400	Rhinitis, oral itch
	6960*	6790	6780	Incompatible	6260	6670	6640	Rhinitis, itch
V. P.	5539*	5523	4948	Incompatible	4823	4815	4515	Hives, asthma, hay fever
	4157	4074	4512	Compatible	4201	4257	5006	None
P. H.	11230	10910	10187	Incompatible	10276	10488	8755	Rhinitis, indigestion
	10200	10100	9940	Compatible	10128	9863	10125	None
R. D.	5737	6125	5930	Incompatible	5685	5416	5514	Hives
	5145	4368	4956	Compatible	4606	4889	5722	None
A. F.	3685	4383	4518	Incompatible	4378	4112	4390	Dermatitis
	4369	4602	4862	Compatible	4722	4962	4815	None
E. D.	9675	8500	9087	Incompatible	8162	7140	8737	Hives
	8120	7160	6880	Compatible	7610	5747	6756	None
R. D.	10125*	8350	8363	Incompatible	8975	8650	9700	Hives
	6395	6231	7405	Compatible	6539	6506	5266	None
R. S.	6147	7506	8445	Incompatible	7250	7260	7260	Hives
	7350	7166	6740	Compatible	7120	6706	7026	None
	6107	6507	5528	Incompatible	6935	5694	5930	Hives
E. P.	3435	4024	3874	Incompatible	3663	5181	5228	Oral itch; canker
	4001	3636	3630	Compatible	4336	3896	4534	None
M. P.	7510	8530	8760	Incompatible	8880	7439	8225	Dermatitis
	3730	4124	4285	Compatible	4656	5067	5533	None
A. C.	6610	5599	6100	Incompatible	6660	6433	5787	Hives
	4922	5095	4808	Compatible	4875	5048	4242	None

*Initial count excluded from calculations because of inadequate rest prior to onset of test.

GROUP IV

ALLERGIC CASES WITH ACUTE SYMPTOMS WITHIN 70 MINUTES AFTER EATING INCOMPATIBLE FOOD

NAME	PREPRANDIAL WHITE COUNT			TEST FOOD	POSTPRANDIAL WHITE COUNT			SYMPTOMS INDUCED BY TEST
	1ST	20 MIN.	40 MIN.		20 MIN.	40 MIN.	70 MIN.	
A. R.	7356	8420	8400	Incompatible	10400	8210	9320	Headache, hives, colic
B. W.	5551	5361	6772	Incompatible	5251	5490	4741	Vomiting
	5432	4822	4902	Compatible	4963	4963	4951	None
P. H.	8500*	8012	7862	Incompatible	7554	7656	7788	Oral itch, nausea vomiting
	7530*	7594	7522	Compatible	7247	7005	7097	None
H. B.	7014*	6764	6523	Incompatible	5930	6235	6581	Asthma
	6225	6469	7300	Compatible	6344	6619	6544	None
V. P.	6217	5666	5731	Incompatible	5028	5159	5200	Hives, asthma, hay fever, edema
	4157	4074	4512	Compatible	4201	4257	5006	None
H. B.	7199†	7065	7758	Incompatible	8473	8241	8291	Vomiting, colic, diarrhea
	10100†	9475	7450	Compatible	10125	9813	10787	None
	10900†	10013	9837	Incompatible	9863	9787	11250	Vomiting, colic, hay fever
	8270†	8020	8863	Compatible	10150	12125	12588	None
	9925†	9938	9400	Incompatible	10250	14300	14325	Vomiting, colic, diarrhea
R. H.	5550	5937	4913	Incompatible	5450	5037	6200	Dermatitis, oral itch
	5275	4963	4713	Compatible	5537	5250	4975	None
A. S.	5892	5958	5567	Incompatible	5208	8140	6610	Nausea, edema
	7060	7290	6630	Compatible	5858	6301	6630	None
	6575	5425	5475	Incompatible	4808	6417	6092	Nausea
	6950	8470	7840	Compatible	7850	8140	7670	None
	6175	6700	6017	Incompatible	5883	5358	5840	Nausea
	10060*	7920	7700	Compatible	7055	7990	8580	None

*Initial count excluded from calculations because of inadequate rest prior to onset of test.

†Entire series of 6 counts eliminated from all calculations because of emotional instability of the subject.

Table V indicates that the group *oscillation,* measured by the standard deviation, was not significantly altered by the injection of either ragweed extract or normal saline solution. Significance was judged by the use of Snedecor's modification of Fisher's "z" test.

In this small group, therefore, the injection of ragweed extract with its generalized allergic reactions in all but one of the patients, did not influence the leucocyte level or oscillation significantly, the behavior being similar to that associated with normal saline injections in the same individuals and also to that of normal subjects before and after ingestion.

Peripheral leucopenia, due to the accumulation of polymorphonuclear leucocytes in the lung, is characteristic of anaphylactic shock in

GROUP V

RAGWEED HAY FEVER PATIENTS GIVEN LARGE SUBCUTANEOUS INJECTIONS OF RAGWEED EXTRACT

NAME	PREINJECTION WHITE COUNT			INJECTION	POSTINJECTION WHITE COUNT			SYMPTOMS INDUCED BY TEST
	1ST	20 MIN.	40 MIN.		20 MIN.	40 MIN.	70 MIN.	
H. B.	7655*	6132	5942	Saline solution	6784	6432	6616	None
	9625*	7160	7090	Ragweed extract	7340	7250	6697	Rhinitis, asthma
B. U.	4831	4612	4705	Saline solution	5469	4991	5826	None
	6023	6189	5939	Ragweed extract	5823	6872	5981	Rhinitis
N. H.	8110*	7170	7400	Saline solution	7310	7990	7700	None
	8390*	7900	7910	Ragweed extract	7210	6720	7400	Asthma, rhinitis
J. C.	6639	6543	6395	Saline solution	6150	7566	6002	None
	8780	6872	8370	Ragweed extract	8720	8360	7720	Asthma, hives
H. B.	7980	6246	6721	Saline solution	6847	8062	6928	None
	6806	5935	6565	Ragweed extract	6528	6524	6172	Rhinitis
R. F.	8120	8400	8990	Saline solution	8250	7760	7730	None
	5747	5974	5281	Ragweed extract	6826	6307	7053	None

*Initial count excluded from calculations because of inadequate rest prior to onset of test.

the dog (Webb[50]). If general reactions induced by the injection of pollen extract in sensitive individuals are anaphylactic, some tendency toward leucopenia might have been expected in the patients of Group V. In no instance did the counts drop from the corresponding fasting average by 1,318 cells or more, although symptoms were delayed until near the end of the test period in some cases. Although the experiment involved a small number of subjects, it suggests that the allergic reaction per se is not associated with alteration in the leucocyte count. It thereby supports our previous contention that the nausea, vomiting, and abdominal pain experienced by Group IV after incompatible food were the source of the increased oscillation noted exclusively in this group.

SUMMARY

The leucopenic index test fell into disuse as an aid to the diagnosis of liver dysfunction, pregnancy, epilepsy, and mental disease because inadequate allowance had been made for errors of method and for physiologic oscillation of the leucocyte count. In investigating the use of the leucopenic index test in food-sensitiveness, we have attempted to control these factors by revision of the technique of the test and by studies upon normal subjects in addition to allergic individuals.

Leucopenic index tests have been performed with a modified technique upon 19 non-sensitive subjects, the level and fluctuation of their white counts before and after ingestion being compared with the corresponding behavior of groups of food-sensitive patients in conjunction with the ingestion of compatible and incompatible food.

In neither the non-sensitive group nor the allergic groups was the average *level* of the leucocyte counts significantly altered from the fasting level, at twenty, forty, or seventy minutes after ingestion of compatible or of incompatible food. The leucocyte *oscillation* was likewise unaltered by ingestion, in all groups except the one (Group IV) in which explosive symptoms occurred during the course of the blood counts; in such cases, blood counts could not be needed for diagnosis, anyway. Since the leucocyte count was not significantly altered by the development of such typical allergic symptoms as asthma, hay fever, and urticaria in the small group of ragweed-sensitive patients injected with ragweed extract, it is fair to assume that the gastrointestinal disturbances in Group IV explain the increased fluctuation in this group.

Attempt was made to estimate the degree of fluctuation in the six white cell counts taken over a period of about two hours in normal subjects. The standard deviation calculated for our 19 normal subjects under controlled conditions was found to average, for 39 leucopenic index tests, ±511 cells; the coefficient of variation, ±7.3 per cent; and the leucocyte level, 7,000 cells. Multiplying the standard deviation by the factor 2.58, one obtains a result of ±1,318. Hence, under similar circumstances, analogous subjects would be expected to normally show upward or downward fluctuations of as much as 1,318 cells from their mean leucocyte count only once in a hundred trials. It was found that the incidence of this degree of leucopenia was no more frequent among our allergic individuals who ingested incompatible food than among the allergic or normal subjects who ingested compatible food.

CONCLUSIONS

1. Experiments with the leucopenic index test upon a group of non-sensitive subjects failed to support the contention that leucocytosis is the normal response to ingestion, since neither the level nor the fluctuation of the total white count during a seventy-minute postprandial period was significantly changed from the corresponding fasting period.

2. A standard deviation of ±511 leucocytes (equal to ±7.3 per cent of 7,000 cells, the approximate average of all the counts) was found in 6 successive total leucocyte counts obtained on each of 19 non-sensitive subjects. A fluctuation of 1,318 cells or more up or down from the mean total count would be expected to occur in analogous individuals, therefore, under our test conditions only once in a hundred

trials by chance alone. Using this figure as our criterion for normal fluctuation, it was found that about the same small proportion of normal subjects showed this degree of leucopenia after ingestion as did the food-sensitive patients after either compatible or incompatible food was ingested. In short, *postprandial leucopenia was not found to be an index to incompatibility* in our experiments.

3. The development of asthma, hay fever and urticaria in a small group of ragweed-sensitive patients given ragweed extract subcutaneously was not associated with significant change in the leucocyte level or fluctuation.

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THE LEUCOPENIC INDEX*

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INTRODUCTION

SINCE it was first described in 1920[1] as the "hemoclastic crisis," the "leucopenic index test" has been advanced as an aid in the diagnosis of many clinical syndromes. Goodman and Connery[2] who reviewed the literature in 1930, proved the test to be an inconstant one in liver disease. In 1932, Power and Pettit[3] reviewing the literature, chiefly English, found the test to be fallacious in mental disease although a previous report by Robertson[4] had stated that 94 per cent of the cases of dementia praecox had postprandial hemoclastic crises. Since 1931 the test has again been brought forward by Denny,[5] Gay,[6] Rinkel,[7] Sullivan,[8] Vaughan,[9] and Zeller[10] as a means of detecting food allergy and also inhalant allergy, Zeller,[10] in patients who have intractable allergic conditions but whose skins react slightly or not at all to their supposedly allergenic foods. It is the purpose of the present paper to examine each step in the process of taking leucocyte counts, regarding it first from the point of view of the literature, second as performed for the leucopenic index in the papers mentioned, and lastly as we have found it in our own work. This, it is hoped, will put the test in its proper perspective in the armamentarium of the practicing allergist.

The authors realize the magnitude of the investigation and will attempt to limit themselves to the pertinent data, referring the reader by means of the bibliography to sources of more detailed discussion of the issues involved. The complexity of the theoretical aspects of the subject and the interrelationships of the many factors involved preclude, however, the publication of a brief paper merely listing our findings. The matter has become so controversial that as much as possible of the evidence must be weighed before final judgment on the utility of the test is passed.

THE SUBJECTS

The patients reported in the early morning in the fasting condition. No ascertainable emotion, fatigue, or excitement preceded the tests. In this manner we were able to fulfill the requirements of Zeller[10] who

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states that with patients in this condition the successive counts show "not more than 5 per cent error."

The patients were kept in a recumbent position for all the experiments except as noted. They remained as motionless as possible but without strain. They rested for fifteen minutes or more before the first count was taken. The room was at a comfortable temperature and with the lights dimmed. No conversation or smoking was permitted although when the former did occur it seemed to have no ascertainable effect upon the subsequent counts. Such cases were, however, not included in our series. In regard to body position and blood counts the literature abounds in conflicting reports.

TABLE I

LEUCOCYTE COUNTS ON 10 SUBJECTS TO DEMONSTRATE THE EFFECT OF POSTURAL CHANGES AND ACTIVITY*

CASE NO.	LEUCOCYTE COUNT WHEN RECUMBENT	AFTER SITTING ONE MINUTE	DIFFERENCE	AFTER WALKING FIFTEEN MINUTES	DIFFERENCE FROM RECUMBENT POSITION	DIFFERENCE FROM SITTING POSITION
1	6225	6987	+ 762	7375	+1150	+ 388
	5525	6025	+ 500	7825	+2300	+1800
2	4729	4500	- 229	5225	+ 496	+ 725
	4760	4780	+ 20	5920	+1160	+1140
3	7472	6716	- 756	7345	- 127	+ 629
	8018	7454	- 564	8000	- 18	+ 546
4	7345	6200	-1145	7345	0	+ 945
	7781	6676	-1105	7166	- 615	+ 490
5	4500	6776	+ 276	4922	+ 422	+ 146
	5713	6492	+ 774	4522	-1191	-1970
6	5885	6584	+ 699	6153	+ 268	- 431
	6200	6766	+ 566	6800	+ 600	+ 34
7	6584	6683	+ 99	7690	+1106	+1007
	7654	6014	-1640	7818	+ 164	+1804
8	5082	5012	- 70	6750	+1668	+1738
	5587	5853	+ 266	6476	+ 889	+ 623
9	4717	5025	+ 308	6507	+1790	+1482
	5587	5450	- 137	6830	+1243	+1380
10		After sitting up for 15 min. 4400				
	4541				- 141	
		After sitting up for 15 min. 5208				
	5025				+ 183	

*Totals given represent the number of cells per cubic millimeter.

Hasselbalch and Heyerdahl[11] and also v. Liebenstein[12] have reported inconstant effects from postural change. Burker (quoted by Garrey and Bryan[13]) and also Garrey and Butler[14] have stated that higher counts were found in erect as compared to recumbent patients although Jorgensen and Jacobsen (quoted by Garrey and Bryan[13]) had stated that the higher counts were present in recumbent subjects. Ellermann and Erlandsen[15] presented evidence that changes in the blood count did occur with changes of posture but were gone in ninety seconds. Bryan, Chastain, and Garrey[16] reported that no constant change was apparent after five minutes.

Vaughan[9] replying to criticism (J. A. M. A.[17]) found fault with the authorities quoted because their subjects had either reported in the afternoon, had eaten, or were at their usual laboratory work. Sullivan,[8] however, mentions that Vaughan's patients (in contradiction to those of Zeller) were permitted to go to restaurants for

the test meal. Denny[5] permits his patients to sit, to read, and to walk but not to talk or smoke. If these variables are grounds for criticism of the work of physiologists they should also be used in judging the work of other allergists who use the test. By having our subjects fasting and recumbent we felt that these variables, if they were variables, could not be used to question our conclusions.

In order to test the validity of such criticism, the effect of postural changes on blood counts was studied in ten patients on whom twenty series of counts were done, and by the technique fully described below (Table I). The subjects, all apparently normal, fasting individuals on whom ten leucocyte counts were being taken at fifteen minute intervals, were permitted to sit up one minute before the ninth count and to walk for the fifteen minutes preceding the tenth count. Each subject had, therefore, been resting for two hours or more. The tenth patient sat up for fifteen minutes as shown.

It should be noted that all but three of the counts are below the 7800 cells postulated by Garrey and Butler[14] as "basal." When the subjects sat up about half or eleven of the twenty blood counts seemingly rose from +20 to +774 cells, the average rise being 428 cells. The two counts shown for each case were, however, taken simultaneously and demonstrate the pitfall of taking a single count in research work of this type. Five of the ten subjects showed a rise by one count and a fall by the other. Of the remainder about half showed rises in each count and two showed consistent falls. But had all the cases had counts all rising or falling within the extreme limits shown it would have meant little, if anything. As described below, slight differences in distribution would account for differences of this magnitude. It should be noted in passing that ten of the twenty counts show greater variation than 500 cells, a number considered significant of food compatibility by Gay[6] and Rinkel[7] who are quoted also by Vaughan although only two, both in the same patient (Case 4), fell beyond the 1000 cell level postulated by Vaughan[9] who states, however, that 800 cells is the "border-line."

On walking, seven of the subjects in nine of the eighteen leucocyte counts maintained their leucocyte count rise or showed greater leucocytoses, and four of the leucopenias became leucocytoses. The two subjects in whom the leucocyte level had fallen when they had assumed the erect position were still acting consistently, their new levels still lying below those taken when recumbent. Compared to the leucocyte count levels observed when the subjects were recumbent the rises vary from 164 to 2300, only one total reaching to three times the standard deviation for the experiment, eight of the eighteen rising over 1000 cells. Taken literally, had these subjects had food, the mere act of rising to walk to the technician or climbing the stairs when returning from the restaurant would have given a leucocyte count acceptable by definition as indicative of a food response although in actuality the magnitude of

difference is not really sufficiently great for us to say that it is due to change in posture or to activity except that it would seem to show a trend toward a rise under these circumstances. Reference to Table VI will show the behavior of the leucocytes in these patients previous to their change in position.

THE BLOOD

The following technique was used for all the experiments performed. The blood was drawn from the pulp of the finger, the hand being held at about the level of the body. Each finger was taken in turn, beginning with the thumb. The finger was cleansed with alcohol and punctured with an automatic stylet which penetrated to the same depth, 3/16 to 1/4 inch, for all the counts of any one subject. There was no method used for estimating the effect of the pain of the puncture on the blood count of the patient although it has been shown by Bryan and others[16] that the mere threat of a finger stab will cause vasomotor changes.

The stab almost always produced free flowing blood. When this did not occur gentle pressure was applied to the proximal phalanx of the finger. We did not at this time corroborate the fact that there is an initial vasoconstriction which passes off in twenty to forty seconds and is followed by a vasodilatation but it was accepted, perhaps erroneously, as a fairly constant factor, the first drops of blood being discarded. But in order to be certain that there was no great difference we examined ten patients on whom we made two hundred and ten observations, other conditions being controlled as described above and below. The first drop was taken after blood was flowing freely and then the second. The pipettes and chambers had been checked as below. In Table II the results of only eighty observations are given since all gave consistent results for the averages of ten drops of blood for each case.

The difference is not great and suggests that there is a higher count in later drops although the greatest was about one half the standard deviation. The variation is of the usual order of magnitude as seen

TABLE II

DEMONSTRATION OF THE DIFFERENCE BETWEEN DROPS OF BLOOD TAKEN IN SUCCESSION*

CASE NO.	MEAN OF EARLIER DROPS	MEAN OF LATER DROPS	DIFFERENCE	ODDS	EFFECTIVE STANDARD DEVIATION
1	4808	5234	+426	30:1	614
2	4794	5313	+519	36:1	638
3	4132	4450	+318	23:1	435
4	6022	6275	+253	8:1	566

*Totals represent cells per cubic millimeter.

throughout the experiments and from these cases one would infer that it is unnecessary to consider the drop after the blood is flowing freely. This factor should, however, be kept constant and mentioned when supposedly causal phenomena in blood changes are presented. Ellermann and Erlandsen[15] stated that successive drops in nervous people showed greater variation than those in phlegmatic people. We were not able to substantiate this in our most vasolabile patients. All of the above counts were below the "basal 7800 cells." The significance of the odds mentioned in Table II will be described below.

THE PIPETTES

The pipettes used were of the Trenner type and where noted, of the Adams type, all certified by the Bureau of Standards. The Trenner pipettes were permitted to fill by capillarity and the blood then diluted with 2 per cent acetic acid. Between observations the pipettes were washed with water and then with ether and dried by an air suction apparatus. The pipettes were shaken manually for three minutes with a combined tilting and rolling motion.

The effect of this on distribution has been analyzed by Garrey and Bryan.[13] We did not use the mechanical rotor recommended by these workers although its use might have given slightly more accurate results. Denny[5] who found the leucopenic index useful in allergic diseases, states that in his experiments the pipettes were shaken "a minimum of sixty times." This would represent about thirty seconds, about one-sixth of the time supposedly required to achieve equal and thorough distribution of the cells, although Vaughan[9] states that an even distribution is most important and requires hand shaking of the pipette for three minutes.

Since so many of the factors are interrelated it is difficult if not impossible to check the error due to pipettes alone. It would have been necessary to use a single sample of the blood, a single chamber and to eliminate the other sources of subsampling error discussed below. We had, however, taken the earlier and later drops as described above and on analysis of 210 observations found the difference between them slight. These drops were taken with two pipettes used always in the same order. The partial error due to the pipettes could not be greater than the combined error due to all the factors involved and since this was not great, could be taken to mean that the pipettes were sufficiently accurate for the purposes of the experiment. Exception could be taken that since the later drops showed higher counts, the truth might be that the second pipette was always measuring higher than the first. But the difference is not sufficiently great. With reference to Table II, taking the cases chosen in order, the successive effective standard deviations were as shown, fairly low. The odds for a rise or fall for these values have been reduced to whole numbers. These have been taken from McEwen's tables[19] for the fluctuations due to random sampling and based on the probability that the ratio of the deviation, x of the mean of a sample, will lie between the limits of minus infinity and +Z. With the highest odds being 36:1 while 100:1 was accepted as the borderline of significance, the pipettes were acceptable. It should be noted that the difference is dangerously close to that accepted as significant in food allergy. Despite the small magnitude of variation, the crucial experiments on the allergic patients described below were all performed with but one pipette.

THE COUNTING CHAMBERS

The chambers were of the Thoma type with the improved Neubauer ruling, all certified by the Bureau of Standards. Detailed work on the accuracy of counting chambers has been done by Jaffe[19a] and also by Power and Pettit.[3]

These latter workers, comparing one chamber with another, took 100 subsamples and for the Thoma-Zeiss gave the error as varying from 0.5 to 22 per cent, for the Neubauer chamber as 0.25 to 24 per cent, and the Fuchs-Rosental as 0.026 to 9 per cent. Ponder and others[18] stated that in their experiments when counting 800 cells the difference between the highest and lowest counts was only 6.5 per cent of the mean but when comparing one side of the chamber with the other there were differences between counts of 18.3 per cent, 26.6 per cent, and 33 per cent, these differences being due to distribution. In his discussion of the reliability of the leucopenic index, Vaughan[9] states that "At least 200 cells are counted (eight 1 mm. squares) in each determination." No mention is made of the type of chamber, its accuracy, or the situation of the squares counted although all are necessary if the work presented is to have fair judgment. This factor will be discussed further below.

TABLE III

DEMONSTRATION OF THE DIFFERENCES BETWEEN HEMOCYTOMETER CHAMBERS*

EXPERIMENT	CHAMBER 1	CHAMBER 2	DIFFERENCE	EXPERIMENT	CHAMBER 1	CHAMBER 2	DIFFERENCE
1	6173	6829	+656	5	6630	6536	− 94
2	7144	7088	− 56	6	7179	6990	−189
3	5966	6503	+537	7	6362	6496	+134
4	4303	4173	−130	8	5417	5486	+ 69

*Totals represent the number of cells per cubic millimeter.

Listed in Table III are observations made on sixty-eight leucocyte counts used to judge the accuracy of our counting chambers. In Experiments 1 to 4, three counts were taken at fifteen minute intervals on subjects under basal conditions and with one pipette as described above. In Experiments 5 to 8, five counts were taken in the same manner except that food had been given before the first count as described below. Since only one pipette was used, and at this time, no conclusions drawn as from one set of totals to the other, the patient's state is not, in any way, germane to the experiment. These facts are mentioned at this time because the table will be referred to again as interesting from another point of view.

It will be seen that four of the series showed rises and four showed falls in the cell counts, with two lying above the 500 cell level. We therefore accepted the chambers as sufficiently accurate as used with our technique and for the purposes of the experiment. The magnitude of the individual counts and the relationship between the experiments

will be discussed below. As usual, despite the slight difference, a single chamber was used throughout the crucial experiments.

THE LEUCOCYTE COUNTS

One of the chief factors in the accuracy of leucocyte counts is the number of cells counted.

Ponder[18] has shown that in counts based on 200 cells the error was three times as great as when 800 cells were counted, the error then being as high as 2.3 per cent. Vaughan, quoted above, states that he counts 200 cells. This, if all other error were eliminated, would represent more than 7 per cent or 700 cells in a total count of 10,000. The standard deviation for the experiments is not given but if greater than this figure, then twice times the standard deviation would invalidate almost all of the data presented. Denny[5] states that "following special experimental work in determining the coefficient of variation while counting 8 sq. mm. (400 cells) and simultaneously counting 16 sq. mm. (800 cells)" he decided to count 16 sq. mm. It is evident that in a total count of 5000 this represents 400 cells so that the coefficient of variation would still be great. Later papers by Gay, Rinkel, Sullivan, Zeller, and Vaughan either make no mention of the number of cells counted or state that eight squares are counted or 200 cells.

In order to be certain of the question of distribution some 740 leucocyte counts were done on forty patients. The experiment was designed so that all the cells in a sample could be counted, the technician noting the number of squares when she reached within 50 cells of 250, 400, 700, 1000, 1400, 1800 cells and then a total of 54 squares no matter how many cells had been counted. The cell count was taken for whole squares and the results calculated as though the count had ceased at each of the levels chosen. The best ten subjects were chosen for Table IV as indicative of the highest accuracy we would have been able to achieve had we limited ourselves to counting 200 or 400 cells. A number of cell counts based on 250 cells were done so that we could determine how few approached the true level. Some did approximate it and might, by good fortune, have been chosen but the majority were unfit for research purposes. For this reason, except as noted, all our counts were based on 32 squares, this source of error then being minimal, since we counted four or more times the number of cells used in doing the first work on the leucopenic index test as shown by the papers quoted.

The remaining thirty subjects showed counts which varied up to 4000 cells, the highest and lowest always occurring in the 200 to 250 cell counts. In Cases 1, 6, and 7, the 400 and 600 cell counts represent the average of the first 200 cells of each of two and three shakings, respectively. All the subjects excepting Case 5 were below the basal level. It is obvious that when the closest counts as taken on forty subjects vary by 654 cells as taken from a single sample of blood and that when thirty-four out of forty subjects show a variation of more than

1000 cells and as high as 4000 cells, then any work postulating a causal relationship when utilizing 200 cell counts should be viewed with grave suspicion. There is sufficient reason why this should be true. The results given in Table IV can be predicted from our knowledge of the mathematics of distribution of particles. If this is a true Poisson series and a sample of blood containing 10,000 cells were to be distributed over 200 squares of a counting chamber, the cells would distribute themselves as follows, using whole numbers.

TABLE IV

ACCURACY OF LEUCOCYTE COUNTS ON NORMAL SUBJECTS WHEN 200 TO 1400 CELLS ARE COUNTED*

	CELLS COUNTED								
CASE	200-250		4-500	6-700	12-1400	54 SQUARES	HIGHEST	LOWEST	DIFFERENCE
1	5375	5500	5412	5408	5534	5510	6029	5375	654
	5475	5525	5513	5669					
	5375	6029							
2	6867	7300	7083	7233	7261	7074	7533	6833	700
	7533	6833	7183	7288					
	6743	7100	6907	6894					
3	6900	7600	7116	7144	7133	7268	7778	6900	878
	7233	7778	7033	7533					
	7100	6967	7416						
4	5000	5400	4744	4938	4911	5004	5400	4511	889
	4511	4622	5082	4895					
	5250	4933	5200						
5	9600	8920	9260	9067	9040	8785	9600	8680	920
	9160	9440	9000	9013					
	8680	8800	8740	8973					
6	6371	6229	6243	6288	6083	6159	6371	5450	921
	5450	6200	5813	5945					
	6114	6229							
7	3488	4600	4044	3837	3914	3914	4600	3488	1112
	4044	4178	4111	3977					
	3467	3711							
8	6556	6600	6585	6770	6764	6775	7233	6086	1147
	6486	6900	6677	6757					
	6086	7233	6615	6800					
9	6800	7033	5842	6066	6053	6007	7033	5743	1290
	5829	6514	6230	6080					
	5743	6057	6461	6320					
10	6543	7167	6867	6855	6902	7046	7833	6543	1290
[illegible]	6800	7833	6830	6947					
[illegible]	7733	7600	7783	7722					

*Totals represent cells per cubic millimeter.

It might be objected that a chamber on which the distribution seemed faulty should be discarded. On the other hand, this very chamber might most closely approximate the truth and in any case we have no mathematical right to be so arbitrary. It is again important to note that the differences found on single samples of blood as shown in Table IV are again of the same order of magnitude as that considered significant by workers using this test in the solution of problems in allergy and that independently of technique, by the laws of distribution alone,

as shown in Table V many of the results could be achieved and not once but again and again. In reference to the repetition of the results it has been stated (Vaughan[9]) that repeated tests have been run with the same foods in quite a number of individuals. In roughly 50 per cent the index remained consistently the same but in 50 per cent there was a change from positive to negative or vice versa. This is singularly illuminating. It is exactly what one would expect from the operations of the laws of chance having the same odds for a successful repetition as the toss of a coin.

TABLE V

DEMONSTRATION OF A POISSON DISTRIBUTION APPLIED TO LEUCOCYTE COUNTS*

NUMBER OF SQUARES	CELLS PER SQUARE	TOTALS	NUMBER OF SQUARES	CELLS PER SQUARE	TOTALS	NUMBER OF SQUARES	CELLS PER SQUARE	TOTALS
0	30	0	8	44	352	6	58	348
0	31	0	9	45	405	5	59	295
0	32	0	10	46	460	4	60	240
1	33	33	11	47	517	3	61	183
1	34	34	11	48	528	3	62	186
1	35	35	11	49	539	2	63	126
2	36	72	11	50	550	2	64	128
2	37	74	11	51	561	1	65	65
3	38	114	11	52	572	1	66	66
3	39	117	10	53	530	1	67	67
4	40	160	9	54	486	1	68	68
5	41	205	8	55	440	0	70	0
6	42	252	8	56	448	0	71	0
7	43	301	7	57	399	0	72	0

*Totals represent cells per cubic millimeter.

SERIAL LEUCOCYTE COUNTS IN NORMAL FASTING SUBJECTS

We had now given consideration to the patients' state and position and to the time of day. We had also given thought to our pipettes, chambers, technique, and counts. Our next investigation concerned serial counts on normal subjects. Four physicians and seven technicians reported fasting and, under basal conditions and the technique described, submitted themselves to ten counts at fifteen-minute intervals as taken by two observers (Table VI). On one subject (Case 5) three series of ten counts were taken by three observers. In Case 11 the leucocyte counts were done on two occasions and by one observer. Four of these subjects have been considered in discussing the difference between earlier and later drops of blood and in comparing the pipettes. In those cases we were interested chiefly in the differences between simultaneous counts as measured by different instruments and in the averages for all ten counts with single instruments. We must now consider the successive counts with the differences as seen at fifteen-minute intervals, all other factors being constant. The subjects sat up before the ninth count and walked before the tenth and these totals have therefore been excluded. The ninth and tenth counts were used in Table I.

It will be seen from Table VI that at some point in their series eight of the eleven subjects had a rise or fall, without apparent reason, in their leucocyte counts accepting only those in whom the difference was greater than 1000 cells. In six of the subjects there was a rise in one series and a fall in the other. The greatest differences occur at no definite point in the series. Had food been given, these differences would have been labeled as significant leucocytoses or leucopenias. In these unselected cases, choosing the appropriate point, all the patterns of allergic patients on allergenic foods can be made and yet since the counts come well within twice the standard deviation of the experiment

TABLE VI

SUCCESSIVE AND SIMULTANEOUS LEUCOCYTE COUNTS ON NORMAL SUBJECTS UNDER BASAL CONDITIONS*

CASE NUMBER	SUCCESSIVE COUNTS								GREATEST DIFFERENCE
	1	2	3	4	5	6	7	8	
1	4858	5075	5037	5112	*5100*	*4421*	4700	4729	– 689
	4500	*5680*	5760	5080	5620	4960	5080	4760	+1180
2	*8720*	*7745*	7166	7066	7016	7345	7763	7472	– 975
	8420	7873	8018	7473	*7327*	*8600*	8500	8018	+1273
3	*5914*	*7000*	7600	8060	7083	6983	7309	7345	+1086
	8780	*6850*	7909	8840	8933	8000	7272	7781	–1930
4	4522	4929	5164	5262	5506	*4941*	*5871*	5885	+ 930
	5117	4729	*4800*	*6461*	6553	5713	6185	6200	+1661
5	7100	6866	7666	7509	*8260*	*5900*	6666	6584	–2360
	7745	6883	*6900*	*8580*	6983	7490	8120	7654	+1680
	6866	6766	6933	6566	7820	*6600*	*8000*		+1400
6	4177	4294	4421	*4330*	*5373*	5087	5373	5082	+1043
	5388	4711	4941	*4700*	*5971*	5105	5562	5587	+1271
7	*3291*	*4191*	4525	3733	4158	4041	3908	4800	+ 900
	3808	4358	5062	4608	4058	4250	*4050*	*4838*	+ 788
8	6825	*6312*	*5437*	6025	5937	5925	5487	6225	– 875
	7137	6900	6437	6087	5912	*6362*	*5837*	5525	– 525
9	*5262*	*4377*	4588	4929	5100	5037	4588	4500	– 885
	5125	5012	4766	*5012*	*5475*	5720	5506	5713	+ 463
10	5137	5237	5025	5573	5262	*5533*	*4776*	4717	– 757
	4612	*4522*	*5550*	5830	6100	5612	5825	5587	+1028
11	3675	3887	3562	3562	*3725*	*4306*	3650	4175	+ 581
	4345	4431	4593	4662	4431	*4356*	*5318*	5418	+ 962

*Totals represent cells per cubic millimeter.

(if one excepts four totals) one could have predicted these differences knowing that they were of no significance. Less than 10 per cent of the counts were above the basal level of 7800. The average value for the coefficient of variation for the experiments listed in Table VI was 0.07826.

As a point of interest it should be noted that the greatest differences between *simultaneous* counts in the ten subjects were, respectively, 723, 1255, 1850, 1661, 1071, 1211, 875, 1000, 1213, and 1049 cells. These differences are of a greater order of magnitude than those due to *successive* counts and due probably to differences in instruments but are

not very much greater, none exceeding the greatest difference seen on successive counts and suggesting that the differences between the instruments were small.

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON NORMAL SUBJECTS

Nineteen normal subjects with no ascertainable allergic history, personal or familial, were fed four to six Uneeda Biscuits and one to two glasses of water. The blood counts (Table VII) were done under the conditions and with the technique described above, the food being taken by the subjects in the recumbent position. Twenty-one series of leucocyte counts were taken. In order that these experiments might be given their true historical evaluation the following brief résumé of the previous work is given.

A complete review of the literature up to 1923 was made by Arneth and Ostendorf.[20] These workers concluded that the rise often observed following protein intake could be accounted for by other factors than food. Shaw[21] quoting also Eisenstadt and ten other workers, Smith,[22] Smith and McDowell,[23] Martin,[24] and Zirm and Bauermeister[25] all found that variation in blood counts occurred independently of food. References too numerous to list but equally confirmatory will be found in the bibliographies of the works quoted above. Yates and Batt[26] reviewing the problem in 1932 found no evidence in their own thirty-six cases that there was a postprandial leucocytosis. Applying mathematical analysis to twenty-four papers on the hemoclastic crisis they concluded that almost all of the results given fell well within the limits of error, in one paper over 75 per cent of the data falling into this category.

Garrey and Butler confirmed by Raisky[27] stated that in patients kept under conditions of minimal metabolism the ingestion of heavy meals did not cause the slightest rise in the low basal count. On the other hand, substances other than food have been reported as causing such changes although in some of the papers the same questions of technique might be raised. In 1921 Walterhöfer[28] reported that hot drinks but not cold drinks caused a leucocytosis. Szabuniewicz[29] stated that the approach of the usual mealtime caused a leucocytosis in dogs although the meal was withheld, the blood changes being conditioned reflexes. Garrey and Butler (loc. cit.) confirmed by Tomoyosi Inada[30] concluded that both hot and cold drinks as well as distention of the stomach with air caused an immediate but transient leucocytosis. And merely to illustrate the number of factors real and fanciful to be considered when postulating or examining the factors said to change the leucocyte count, it was reported by Glaser and Buschmann[31] that mere mastication and deglutition caused a leucocytosis, and by Müller and others,[32] and also Tinel and Santenoise (quoted by Merklen and Wolf,[33] that the oculocardiac reflex was associated with a leucopenia. This would suggest that a patient had only to rub his eyes to invalidate an entire experiment.

Workers using the leucopenic index test in allergy have stated (Zeller[10]) that allergic patients on nonallergenic foods show a rise, postprandially, of 1000 to 4000 cells, the count returning to the base line in 100 minutes. This rise is noticeable after forty minutes. Vaughan[9] states that it makes no difference whether or not a postprandial leucocytosis exists because we are not so interested in counts above the base line as below. Compatible foods, however, throughout the text and diagrams of the papers quoted invariably show leucocytoses. Vaughan[9] in defense of the postprandial leucocytosis quotes the work of Jones, Stephens, Todd, and Law-

rence[34] as concluding that there was a postprandial leucocytosis. Analysis of this work, however, shows that the leucocytosis only occurred in nine of the fifteen subjects and then at the hours of the supposedly daily tides. In their monumental work on the subject which should be consulted by all workers on leucocyte counts, Garrey and Butler conclude "in view of the many physiological responses and adjustments associated with alimentation, to deny that any of these can cause changes in the leucocyte count would be unjustifiable. The question needs investigation from many angles."

For the first five subjects we followed the technique of the workers in allergy taking two preprandial and three postprandial counts. Analysis by the mathematical author proved these to be the least reliable of our data even though 32 squares or four to eight times as many white cells were counted as were used by the workers quoted.

TABLE VII

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON NORMAL SUBJECTS

CASE NUMBER	AVERAGE OF PREPRANDIAL COUNTS	AVERAGE OF POSTPRANDIAL COUNTS	DIFFERENCE	ODDS FOR THE SIGNIFICANCE
1	5697	6856	+1159	4:1
2	5166	5472	+316	2:1
3	5065	5733	+668	29:1
4	5572	6394	+882	10:1
5	5028	5547	+519	4:1
6	6734	6514	−220	5:1
7	3347	3322	−25	1:1
8	3609	3929	+320	4:1
9	7078	7298	+220	2:1
10	13578	14208	+630	3:1
11	6838	7781	+942	12:1
12	5934	6499	+565	3:1
13	5817	6091	+274	3:1
14	7035	6889	−146	2:1
15	6871	6964	+93	1:1
16	7892	8428	+536	3:1
17	5268	5349	+81	2:1
18	8201	9076	+875	40:1
19	8784	8084	−700	46:1
	7923	9060	+1137	20:1
	6389	7814	+1425	26:1

In Cases 1 to 5 inclusive (Table VII), the preprandial and postprandial counts were respectively two and three in number; in Cases 6 to 12, they were four and three; in Cases 13 to 15, five and three; in Cases 16 and 17, five and two; in Case 18, they were five and three but the patient sat up during all of the tests. Case 19 represents three sets of counts done on the same subject on different days, six preprandial and three postprandial counts being done. By increasing the number of preprandial counts it was possible to use the subjects, as it were, as their own "controls" and in this manner learn what their "pat-

terns'' were before giving them food. Nor did they disappoint us since each gave at least one large difference in the preprandial counts thereby robbing the differences in the postprandial counts of all meaning. To illustrate this: the consecutive counts for Case 13 were, preprandially 6925, 5931, 5668, 5037, 5525 and postprandially 6231, 5918, and 6125; for Case 14, 6800, 7812, 6731, 6431, 7400 and postprandially 6737, 6837, and 7093; for Case 15, 5856, 6831, 7187, 8231, 6250 and postprandially 6381, 7756, and 6737. In only three subjects, only eight totals were above the basal level.

It might be objected that to give the averages of the two sets of counts results in a false picture, and, in one sense, this is true. The odds for the significance of the difference are therefore given and must be taken to mean that when two cases show a rise or fall of an equal number of cells the one with the greater odds has shown a narrower variation between the individual preprandial counts. In the first case in the table, the difference between the two sets of counts was +1159 cells but the individual counts varied greatly and the odds are only 4:1 but in the nineteenth case the difference was +1137 for preprandial totals that were very close to one another and the odds, therefore, are those of true significance.

Vaughan[9] states that he takes the average of the two preprandial counts and compares each postprandial count with this average. There is no mathematical precedent for treating numerical data in this special manner.

It would not be profitable to analyse these cases further. It would seem that with the technique as described the subjects showed a trend toward a postprandial leucocytosis. But all the average differences observed fell within twice the standard deviation and the preprandial variations were so great when measured between individual counts that the differences between the two sets of counts cannot have great significance. A definite conclusion that there is a trend however slight following food ingestion cannot be drawn from these experiments since analysis of the subjects given in Table VI on each of whom twenty counts were made with the patients fasting shows that when the counts are arbitrarily divided into earlier sets and later sets various conclusions, all of course invalid, may be drawn.

Taking Case 3 in Table VI the second series of counts may be divided into a set of three and a set of five in which instance there is a rise of 319 cells, or else five and three, in which instance there is a fall of 578 cells. In Case 2 in the same table the counts may be divided into two sets again, two and six giving a fall of 928 cells, or six and two, a rise of 108 cells. The second series in the same case taken as five and three demonstrate a rise of 550 cells, or the three and five give a fall

of 119 cells. These are various examples. Other methods of rearranging the data to achieve any desired result will suggest themselves to the more ingenious reader. Taking the data in Table VI in another manner, the counts may be treated to resemble those of Table VII. In all the cases in Table VI the first five observations are treated as though they were preprandial counts. In Cases 1 to 4, 8 and 10 there were three, as it were, postprandial counts, in Cases 5, 6 and 11 four, as it were, postprandial counts and in Cases 7 and 9 there were five, as it were, postprandial counts. It will be seen on consulting the following table (VI A) that the results achieved by treating this imaginary data in this fashion, imaginary since the patients had had no food, in no way differ from those seen in Table VII following food ingestion. It may, therefore, be concluded from the data in Table VII that there is a trend toward a leucocytosis, postprandially, but it is not great and cannot be predicted.

TABLE VI A

SERIAL FASTING LEUCOCYTE COUNTS TREATED AS THOUGH FOOD INGESTION HAD TAKEN PLACE AFTER THE FIFTH OBSERVATION

CASE NUMBER	POSTULATED PREPRANDIAL COUNTS	POSTULATED POSTPRANDIAL COUNTS	DIFFERENCE	ODDS FOR SIGNIFICANCE
1	5037	4617	−420	28:1
	5328	5933	−395	6:1
2	7543	7526	−17	1:1
	7823	8373	+550	17:1
3	7131	7212	+81	1:1
	8262	7684	−578	5:1
4	5077	5566	+489	7:1
	5532	6033	+501	4:1
5	7480	6735	−745	11:1
	7408	7361	−47	1:1
	6890	7300	+410	3:1
6	4519	5139	+620	26:1
	5412	5526	+384	6:1
7	3980	4284	+304	5:1
	4379	4522	+143	2:1
8	6107	5872	−228	3:1
	6615	5908	−707	14:1
9	4851	4765	−86	2:1
	5078	5591	+513	9:1
10	5247	5009	−238	4:1
	5313	5675	+362	4:1
11	3682	4153	+471	28:1
	4492	5147	+655	57:1

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON ALLERGIC PATIENTS AND COMPATIBLE FOODS

Although normal patients behaved as noted in the previous section it occurred to us that allergic patients might by their very nature behave quite differently even though they were given compatible foods. Six food-sensitive patients were subjected to ninety-four observations. On each we took a series of two parallel counts taking three of these pre-

prandially and five postprandially. Two chambers were used but only eighteen squares counted for each chamber (all the squares of both sides, about 900 cells for a 10,000 cell count and others proportionally). These subjects had never, except Case 6, Table VIII, previously given evidence of sensitivity to the foods chosen and as far as we know have not since demonstrated food sensitivity. Since all behaved consistently no further cases of this type were studied.

TABLE VIII

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON ALLERGIC PATIENTS AND COMPATIBLE FOODS

CASE NUMBER	CHAMBER	AVERAGE OF PREPRANDIAL COUNTS	AVERAGE OF POSTPRANDIAL COUNTS	DIFFERENCE	ODDS FOR THE SIGNIFICANCE
1	A	3596	3679	+ 83	2:1
	B	3492	3675	+183	6:1
2	A	5475	5086	−389	32:1
	B	5307	4862	−445	7:1
3	A	5033	5102	+ 69	1:1
	B	5022	5032	+ 10	1:1
4	A	7144	7179	+ 35	1:1
	B	7088	6990	− 98	1:1
5	A	10946	10284	−662	7:1
	B	10262	10743	+481	11:1
6	A	5966	6362	+396	8:1
	B	6503	6497	− 6	1:1

It can be seen without further analysis that the response was, if present, of the usual type and magnitude. Because of the importance of the basic data the individual counts of these patients are given.

TABLE IX

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON ALLERGIC PATIENTS AND COMPATIBLE FOODS:

Individual serial and simultaneous observations

CASE NUMBER	CHAMBER	PREPRANDIAL COUNTS			POSTPRANDIAL COUNTS				
1	A	3486	3755	3444	3711	3377	3510	3810	3988
	B	3266	3721	3488	3721	3688	3455	3810	3699
2	A	5388	5477	5560	4877	5266	4933	5033	5321
	B	5121	5755	5044	*5488*	*4533*	4366	4755	5166
3	A	4910	5233	4755	5022	*4255*	*5655*	5122	5455
	B	5455	*5311*	*4299*	5188	4410	4844	*4655*	*6021*
4	A	7455	*7410*	*6566*	6888	*8010*	*6277*	7255	7466
	B	7766	*7322*	*6177*	7177	6766	7421	6677	6910
5	A	10777	*11622*	*10438*	10132	*11144*	*9477*	10711	9455
	B	10711	10121	9955	10444	11183	10466	10899	10772
6	A	6155	6033	5710	*5711*	*6711*	6222	6722	6444
	B	*6155*	*7433*	*5922*	6277	*6244*	*7110*	*6199*	6655

It will be seen that all of the observations in the fifth case and one in the fourth case were above the basal level, all the rest of the leucocyte counts being below 7800 cells. The sixth case was that of a child who had a history of urticaria following the ingestion of chocolates contain-

ing nuts. He was given some nuts before the third and fourth counts and peanut extract between the fourth and fifth with no undue effect at that time or subsequently. It may be noted in comparing Tables VIII and IX that although the individual counts taken serially often show what are apparently large differences (as italicized) the averages for the series before and after food differ by little, suggesting that these deviations are probably occurring at an equal distance on either side of the mean. And also, despite the differences shown by the two chambers for counts taken simultaneously the averages for the two chambers approximate closely. It might again be objected that by taking averages the individual variations may be lost. In answer it should be remembered that individual counts mean nothing when we are searching for the effects of a stimulus supposedly acting over a period of time. The effect of the trend then overrides any individual variation since each individual variation is also partially affected in the direction of that trend. The rises and falls in blood count occur at any point in the series, before and after food, with one chamber and not with the other, and probably lack all significance however small the series. The successive counts illustrating the greatest differences are italicized in all tables in which this difference seemed significant.

The danger of taking the average of only two preprandial counts as representing the resting count should be noted. In four of the cases the difference in averages when taking three counts is quite substantial although with more blood counts the difference from the mean would become progressively less as the number of observations increased.

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON ALLERGIC PATIENTS TAKING INCOMPATIBLE FOODS

In both the choice and preparation of patients with food sensitivity we discovered many technical difficulties. In the work describing the "leucopenic index test" the subjects appeared to be chosen from those groups of allergic patients who usually do not give skin reactions. If it were true that normal subjects give postprandial rises and food-sensitive subjects give postprandial falls in blood counts it was questionable whether these could be discovered, no less measured by the technique used. If neither premise was true the technique was of little importance. The only possible controlling factor is the patient's history of food allergy and the patient's subsequent course when the food is removed. Allergic patients are, however, notoriously capricious and regarding their subsequent courses the literature is unfortunately meager. Have the subjects had previous remissions or additional treatment either passive as by dust or feather elimination or active as by injection or operation? And also, the fact that the patient improved

by diet alone does not prove the eliminated food as having caused the remission, since some of the patients described by Rinkel[7] were well only when limited to two or three foods. Both partial starvation as well as diets limited to any two or three foods are often associated with remarkable improvement in inhalant and infective cases of asthma. Vaughan[9] states, "There was 81 per cent correspondence between food allergy and the leucocytic response." He mentions 93 per cent accuracy when the test is performed "with utmost care." Our subjects were, therefore, chosen from the group who gave clinical histories and objective symptoms so that known factors could be measured by known technique.

There were, however, additional difficulties. Known food-sensitive cases are not plentiful. Of these again, only certain subjects could be chosen. If the patient is so sensitive to the food that a minute portion causes immediate and striking symptoms the test is dangerous, unnecessary, and inaccurate, first, because the patient is subjected to a fulminating procedure, second, because the information is at hand and requires no further corroboration, and third, because the blood picture if affected might be that of any stage of shock. The effort associated with an attack of asthma and the treatment by adrenalin will both affect the count or by their presence introduce variables which cannot be measured. On the other hand, if the symptoms are too long delayed it cannot be argued (although it is implied in the works mentioned) that the blood count in the first two hours following food ingestion adequately foretells the patient's condition by two weeks (Rinkel[7]). It would seem illogical, although we have no proof for this hypothesis, that the blood count should behave in this fashion especially since we have no analogous physiologic phenomenon. Such cases, those giving immediate or long delayed symptoms have, however, been studied (Loveless, personal communication). The patients chosen for the present experiment were, except as noted, those who gave objective symptoms within two to four hours following the ingestion of the allergenic food. It is necessary to stress the fact that the symptoms were objective because those of the control series in Vaughan's preliminary report[9] were, except for urticaria, entirely subjective.

The preparation of the patient was far from simple and from the published papers (loc. cit.) would seem very complex. Rinkel[7] states that the curve changed in that abstinence from the symptom producing food causes a leucocytosis while indulgence brings on a leucopenia although actually eating the food does not cause symptoms at that time. Vaughan[9] on the other hand, states that a patient may fail to give a leucopenia on repeated indulgence or repetition of the test because the blood is "refractory," the patient being in "allergic equilibrium." Gay[6] agrees with both that his patients become refractory when not fed

the food since he says, "if the food is tested without it having been eaten a few times in the week preceding the count a curve denoting compatibility may be obtained," and also, "the leucocyte response becomes refractory to an occasional previously restricted food until it has been in the diet for some time." If these hypotheses be true any failure on the part of a third worker to achieve similar results can easily be explained away, since the allergenic food will not cause a leucopenia if the patient has had either too much of it or too little. One wonders how the test was ever discovered. It would require such great clinical acumen to do leucocyte counts on patients the very day on which first, the response had become a leucopenia, second, the patient's blood was not "refractory," and third, the symptoms following the food did not appear for some days, that we felt this feat approached the impossible. Nor do the published reports suggest that the problem had been approached with such delicacy. Instead, as a preliminary test, our patients were asked to eat the food twice daily for three days preceding the experiment. Subsequently the foods were eliminated from the diet and then added again and again until the patient rebelled and it was certain that the remissions and exacerbations were due to the specific food. No apology is therefore made for the small number in the series.

Many other factors remain to be weighed. Although little attention is given to the exacting technique of the hematologic aspects of the problem, it is stated in the papers mentioned that it is very important that the patient be given the food in the state, quantity, and temperature it is usually eaten and at the usual rate. One observer, Rinkel,[7] uses a steam cooker. Our patients knew, however, that symptoms arose no matter how much or how little of the food they ate and no matter how slowly or quickly. This was shown to be true by their subsequent courses. Zeller[10] described a leucopenia following inhalation in an inhalant sensitive patient. In this experiment a fasting dog-sensitive patient was left in proximity to a dog. In ten minutes the blood count had dropped 500 cells and in twenty minutes 1400 cells below the fasting counts. One would like to see this experiment confirmed.

We could not discover from the papers mentioned the time limit during which the counts had to be taken. Vaughan[9] states that if the counts as taken at thirty, sixty, and ninety minutes are not satisfactory another at one hundred and twenty minutes is to be taken. Zeller[10] states that the counts should be continued until the curve reflects adequately the nature of the food response. We felt that in our experiments this attitude might have led us astray. In the normal patient we might have kept on taking blood until a leucocytosis of great degree occurred or continued with the allergic patient until a corresponding leucopenia, if any, came along.

It was important to avoid a common fallacy in this work. It is very easy to fall into the error of labelling each patient who shows a leucopenia, such as it may be, as being allergic and the food as being incompatible and then to forget that both of these statements were made by definition and then later, to state that the allergic patients on allergenic foods all averaged a fall in blood counts of so many or so many cells.

The mathematical factors to be discussed are many and only a few will be mentioned. It has been the practice of workers using this test to take only one preprandial count or in some cases, two at ten-minute intervals and to use the average of these for comparison with each postprandial count. However, in taking the average of the preprandial counts there is another factor requiring consideration. Three patients all of whom show a first postprandial count of 4000 may have had two preprandial counts, the first successively 6000 and 6000, the second, 4000 and 8000, and the third 8000 and 4000. In each case the preprandial counts average 6000 cells and the first postprandial count shows a fall of 2000 cells from the average, but no two of the three observations are comparable. Vaughan states that there is often an unexplained drop of 2000 cells between the two fasting counts. It would seem that if this were inexplicable for the preprandial counts it would be equally inexplicable for those taken postprandially.

In some of the reports quoted, the cell counts are so relative that one wonders that any conclusions whatsoever were drawn. The difference of 500 cells is mentioned as being compatible although 500 cells in a 5000 cell count is hardly equal to 500 cells in a 10,000 cell count. An analysis of the data brings to light some striking coincidences. In one of Vaughan's reports[9] in 191 tests 62 per cent corresponded with the skin tests. This is very close to the number that we would have expected to respond in this way independently of all other factors. For each positive skin test the blood count can either show a rise or fall (with the technique given); in about half the cases it corresponds. On repeating the test, 50 per cent of the curves remained the same. This too is the expected percentage. Had this 50 per cent been subjected to a third series we could expect another 50 per cent to remain the same.

These percentages are again seen in Zeller's paper.[10] Of 106 patients tested, fifty-six showed a leucocytosis and fifty a leucopenia. Of fifty-four clinical positives, thirty-seven revealed negative skin tests and decreases in the white cell count of more than 1000 cells. This equals 34.91 per cent of the total cases tested. In this mathematical analysis in which the first number to the right of the decimal point is not a significant figure, we are given two, an accuracy only acceptable if over 10,000 cases had been taken. Gay[6] uses mathematics in the same man-

ner when three of six cases are mentioned as being 50 per cent. Rinkel[7] making no mention of conditions, basal counts, technique, or mathematical error, accepts a 500 cell increase or decrease as sufficient. In Sullivan's[8] "statistical" discussion of Vaughan's cases, statistical terms and methods are not employed. Obvious as the facts may be it should be remembered that as the inaccuracy becomes greater the magnitude of variation becomes proportionally greater but the importance of these variations lessens in equal proportion. The true issue at stake is not that there is a rise or fall in the cell count but whether or not the rise or fall is first, mathematically, second, physiologically and third, allergically, significant. One feels, therefore, that although our work and the literature do not necessarily disprove the leucopenic index test the papers themselves describing the test are utterly unconvincing.

And lastly, to remove the last prop of those who would attempt to repeat the work, Rinkel[7] states that there is "no normal control." Similar curves are found in cases of "seborrhea, acne and trachoma," the important thing being that "in the allergic individual the food that causes a positive response does not provoke symptoms at that time." This would infer not only that nonallergic patients on compatible foods act as do allergic patients on incompatible foods but also that the patients of whom we are most certain clinically are least satisfactory for the leucopenic index test while those of whom we are least certain clinically are most satisfactory for the test. One can recall no other procedure in medicine in which these conditions hold true.

ONE MATHEMATICAL APPROACH TO HEMATOLOGIC PROBLEMS

The following brief summary of one mathematical approach is given. Whenever a "population" varies from time to time, the variation being due to any cause whatsoever, we can calculate the significance of the variations by studying the variations of the means of a number of different observations taken at different times. These means more closely approximate the truth as the number of observations taken increases at each particular time. The differences between means for any specific set of observations are due to errors in sampling. This type of difference is an error which can never be completely eliminated.

When we take the mean of the leucocyte counts of blood samples drawn preprandially and postprandially there is a difference between the two although all the samples are taken from what may be the same or different universes. We wish to discover whether or not an actual physiologic change has taken place, or if it did not take place, to show that the differences that occurred were of such an order of magnitude as to prove themselves independent of food ingestion.

Upon plotting a large number of leucocyte counts for the present experiments we discovered that we were dealing with a universe which was distinctly bell shaped and also followed rather closely the curve for a normal population. We used, therefore, an index developed by "Student"[35] for problems of this type. The index was developed for use on normal populations, but the errors cancel out very well when the universe is bell shaped. When samples are taken from a normal population it is easy to show that the means of a series of samples follow a Bernoulli Distribution. But "Student" showed that the standard deviations of

these individual samples did not follow a normal law but did follow the distributions given below, the first for n being even and the second for n being odd.

$$1.\ y = \frac{N}{(n-3)\ (n-5)\ \ldots\ 3.1}\sqrt{2/\pi}\ \left(\frac{n}{\sigma^2}\right)^{\frac{n-1}{2}} X^{n-2}\ e^{\frac{-n\,x^2}{2\,\sigma^2}}$$

$$2.\ y = \frac{N}{(n-3)\ (n-1)\ \ldots\ 4.2}\left(\frac{n}{\sigma^2}\right)^{\frac{n-1}{2}} X^{n-2}\ e^{\frac{-n\,x^2}{2\,\sigma^2}}$$

Where N is the total number of groups of samples and n is the number of samples per group, x is the standard deviation of the samples and σ the standard deviation of the population. It should be noted that these distributions are nowhere near normal for the small values of n as used in our experiments.

By assuming first, that the means of the sample follow a Bernoulli Distribution and second, that there is no correlation between the standard deviation of the sample and the deviation of the mean of the sample from the mean of the population, it can be shown that the number of times the difference of the mean of the sample and the mean of the population exceeds the standard deviation of the sample follows the distribution given below, the first equation for n being even and the second for n being odd.

$$1.\ y = {}^1/\pi\ \frac{(n-2)\ (n-4)\ \ldots\ldots\ 4.2}{(n-3)\ (n-5)\ \ldots\ldots\ 3.1}\ (1+z^2)^{-n/2}$$

$$2.\ y = \tfrac{1}{2}\ \frac{(n-2)\ (n-4)\ \ldots\ldots\ 5.3}{(n-3)\ (n-5)\ \ldots\ldots\ 4.2}\ (1+z^2)^{-n/2}$$

Where y is the number of observations which have the given deviation, z is thus really the ratio of the difference of means to the standard deviation of the sample. This is very advantageous from an analytical point of view because it does not involve the standard deviation of the population, an obviously unknown quantity. The area covered by each of these curves is equal to 1. They are therefore probability curves.

Thus the probability that the difference of the means divided by the standard deviation of the sample shall lie between minus infinity and any given value of z is $\int_{-\infty}^{z} y\,dz$ using the value of y given above.

This operation can best be carried out by introducing a change of variables $z = \tan\theta$. This gives us the following equation for the probability.

$$P = \frac{(n-2)\ (n-4)\ \ldots\ldots}{(n-3)\ (n-5)\ \ldots\ldots}\left\{\begin{matrix}{}^3/_2\ {}^1/_2\\ {}^2/_1\ {}^1/\pi\end{matrix}\right\}\begin{matrix}(n\text{ odd})\\(n\text{ even})\end{matrix}\int_{-\pi/2}^{\tan^{-1}z}\cos^{n-2}\theta\ d\ \theta$$

Tables for the value of this integral for any n and any z have been prepared by McEwen.[19]

By evaluating this integral and subtracting the value from 1 we obtain the probability of an even greater difference in means occurring. If the probability (not the odds for the significance) is large it indicates that an even greater difference of means could have occurred and still be probable, that is, that the means were calculated from two populations which were equivalent. If, however, the probability is small it indicates that greater differences could not occur and, therefore, the two means were probably not drawn from the same population. Another method of analysis, the Fisher "t" test, was used as a check on about half of our results. The accuracy was in some cases slightly greater but the ease of operation using McEwen's tables, on the one hand, and the increased complexity of the Fisher "t" test, on the other, inclined us to use the present method knowing its limitations. Borderline cases,

those in which the odds approach or exceed 100:1, should be checked by the Fisher "t" test. A hypothetical application of our method may be made as follows:

The preprandial counts were 5037, 4881, 5650, 5125.
The postprandial counts were 5369, 5400, 5718.
Are the two sets of data drawn from the same population?
A: The average of the first series = 5173.
The average of the second series = 5496.
B: The difference of means = 323.
C: The standard deviation of the series

$$\sigma_1 = \sqrt{\frac{(5037 - 5173)^2 + (4881 - 5173)^2 + (5650 - 5173)^2 + (5125 - 5173)^2}{4}}$$

$$\sigma_1 = \sqrt{83{,}398}$$

$$\sigma_2 = \sqrt{\frac{(5369 - 5496)^2 + 5400 - 5496)^2 + (5718 - 5496)^2}{3}}$$

$$\sigma_2 = \sqrt{24{,}876}$$

D: The effective standard deviation of the two series

$$\sigma^2 = \sigma_1^2 + \sigma_2^2$$
$$\sigma = 329$$

E: Calculate z as $\frac{323}{329} = 1.0$.

F: But the number of observations differ in each series, being three and four. We calculate twice and interpolate once for z = 1, n = 4, and z = 1, n = 3. The tables give these values.

z = 1, n = 4 0.9092
z = 1, n = 3 0.8536
but 0.9092 + 0.8536 = 1.7628
$\frac{1.7628}{2} = 0.8814$

G: Subtract 0.8814 from 1.000 = 0.1186 which is the Probability.

H: The odds that there is any difference between the means is 8814:1186 or about 7:1, odds for the significance of the rise.

Conclusion. The two series are drawn from the same population.

The totals in Table X have been treated in the above mentioned manner.

Another case with further treatment but omitting the details would show that for an absolute count of 8,100 cells, based on a 200 cell count, an upper limit of 11,400 and a lower limit of 5,300 cells could be expected, a range of 2,800 cells on either side of the mean lacking all significance and true if all possible error had been eliminated and *a priori before a single cell had been counted.*

Represented in Table X are twenty-nine series of leucocyte counts taken on twenty allergic patients. In the cases marked "A" and "B" the letters represent two different allergenic foods tested with an interval of several weeks between them. In the cases designated as "x" and "y" two observers took simultaneous observations while one food was being tested. The averages for both preprandial and postprandial totals are given, the difference between them and the odds for the significance of the rise or fall. All of the actual leucocyte counts are given in order that future investigators employing other methods of analysis may confirm or contradict our conclusions in these cases. Many more cases than those described were done but omitted not because they showed differences in pattern but because only sixteen squares were

TABLE X

PREPRANDIAL AND POSTPRANDIAL LEUCOCYTE COUNTS ON ALLERGIC SUBJECTS FOLLOWING INGESTION OF INCOMPATIBLE FOODS

CASE NUMBER		PREPRANDIAL TOTALS 1	2	3	4	5	6	7	AVERAGE	POSTPRANDIAL TOTALS 1	2	3	4	5	6	7	AVERAGE	DIFFERENCE	ODDS
1		7025	6675						6800	6137	6981	7231					6783	− 17	1:1
2	A	3078	2696						2887	3303	3282	2932					3172	+ 285	4:1
	B	3633	3783						3708	3812	3745	4687					4081	+ 373	4:1
3	A	5550	5700						5625	6666	6768	6612					6681	+1056	50:1
	B	5750	5693						5724	7666	8633	7558					7953	+2229	21:1
4	A	7112	7175						7144	8317	7925	8000					8081	+ 837	26:1
	B	5206	5843						5525	6568	6512	5412					6164	+ 639	4:1
	C	5575	5893	5587	5712				5692	6262	6362	6525	6531	6368	6725		6462	+ 770	832:1
5	A	3933	4050						3992	4805	3612	4890					4436	+ 444	3:1
	B	3750	3806	3731	3687				3744	4312	4737	4500					4516	+ 772	40:1
6	A	5462	4700						5081	5612	5737	6637					5995	+ 914	6:1
	B	5037	4881	5650	5125				5173	5369	5400	5718					5496	+ 323	7:1
7	x	5988	6244	8566					6933	7166	7766	7288	7884	8444			7710	+ 887	5:1
	y	5234	6977	7110					6440	6799	6988	6821	7644	6716			6994	+ 554	4:1
8		7806	7693	7287	7775				7641	9015	8868	10306					9396	+1775	44:1
9		7631	7637	7481	8025				7694	7206	6593	6532	7756				7022	− 672	16:1
10		5950	5837	5287	6468				5885	5356	5062	5181					5200	− 683	17:1
11		5912	4587	4493	5287	5662			5188	5412	5068	5250	5137				5217	+ 29	1:1
12		8000	6925	6468	7281	8118			7358	7843	7068	7825	7287	6675	7562	6462	7246	− 112	2:1
13		5587	4668	4950	4156	4462			4765	4250	3787	4081	4093				4053	− 712	27:1
14		8500	7666	8066	8016	7916			8033	8035	7758	8378	7866				8009	− 24	1:1
15		6381	6056	4993	5181	4850			5492	5775	5112	5668	5281				5459	+ 33	1:1
16		4468	4356	4281	4600	4925			4526	5250	5012	4631	5462				5089	+ 563	27:1
17	A	9900	9891	8831	8668	9168			9292	9243	10506	10568	10287				10152	+ 860	19:1
	B	9356	8456	8962	9525	9850			9230	10758	11000	11207	12212				11294	+2064	184:1
18	x	10125	7675	6900	7781	9100			8316	8127	8660	8920					8569	+ 253	2:1
	y	10622	8850	8040	8600	9288			9080	8520	9220	7836	9020				8649	− 331	2:1
19		4237	3968	3812	4118	4681	4381		4202	4831	5331	5587	5525				5319	+1117	199:1
20		5318	5206	5112	4787	5581	5309	4743	5293	5318	4900	5125					5114	− 179	3:1

counted or because of other variables such as emotion, activity, conversation, immediate symptoms or too long delayed symptoms, or psychologic factors. The blood counts in these omitted cases were no different than those listed but the description of the circumstances for which each one was excluded would have made a lengthy paper even more lengthy.

It should be noted that it is impossible in our Tables as well as in those given in all previous reports without exception, to determine from the totals given whether or not food was ingested, when it was ingested, and whether or not it was compatible.

The majority of the cases (all but eight) show a rise in the postprandial counts. In five cases it is above 1000 cells. In only three cases was the rise of mathematical significance. Not one patient showed a mathematically acceptable leucopenia. The twenty-one leucocytoses were neither great nor predictable.

It might be inferred that although no single patient showed a postprandial leucopenia following the ingestion of incompatible foods, the patients as a class could show some trend. Taking the last two preprandial and the first two postprandial totals the averages for seventeen cases were, respectively, 5685 and 6077. The rise was 392 cells with odds for significance of 4:1. In these cases there was no trend.

As a matter of interest in order to test the "leucopenic index test" still further we made successive leucocyte counts upon a patient extremely sensitive to celery. The blood counts were done as described above. The patient knew of his sensitivity, and had not eaten celery for some years. The successive preprandial totals were 4937, 5231, and 4881. He ate two stalks of celery and the successive totals were 4600, 5331, 4650, and 3900. We gave him three more stalks. The counts were 3800 and 4200 when he broke out in massive wheals covering the entire body. We stood by with adrenalin but the patient expressed his desire to cooperate. Successive counts were 4737, 4093, and 3918 before obstructed respiration made adrenalin necessary. Adrenalin, twenty-four minims, in three doses brought relief and raised the leucocyte level to 8787. Despite the great degree of somatic response the blood picture had remained similar to that of the majority of our normal patients, and it was impossible to discover from examination of the totals the point at which celery had been given or symptoms had occurred.

At this time Table III should be consulted. The first four cases are allergic subjects, the postprandial counts being labelled as though separate experiments. Each should be taken as one case reading across the page. The blood counts show greater differences between simultaneous observations than on successive observations. The patients behave as though they had had no food rather than a food which causes symptoms.

The cases in Table X require little analysis. Case 17 should be noted, the subject behaving in the same way to two allergenic foods, the tests being taken weeks apart. Only three subjects show any significant changes. The leucocyte counts are just as "chaotic" in their patterns as

one would expect them to be. Any defender of any theory relating physical causes to hematologic effects could find and select cases in support of his hypothesis. But when the errors inherent in sampling and subsampling are considered, it is quite comprehensible that such conclusions could be drawn from a literal acceptance of leucocyte counts but it is also obvious that these conclusions are invalid.

CONCLUSIONS

1. Each step in the technique of taking leucocyte counts was standardized for blood drawn under basal conditions.

2. Differences of a small order of magnitude were found to exist between different observers, instruments, drops of blood, and change of position.

3. Variations of the same order of magnitude were found to be present when comparing subsamples taken simultaneously or successively on normal fasting subjects, on normal subjects preprandially and postprandially, on allergic patients preprandially and postprandially, following the ingestion of either compatible or incompatible foods.

4. No adequate proof was found for the existence of either a normal postprandial leucocytosis or an anaphylactic postprandial leucopenia.

5. The techniques used previously in this work were shown to be questionable in accuracy. The conclusions drawn were mathematically untenable. In our series of over 2000 leucocyte counts we found no physiologic, pathologic, or mathematical justification for the use of the leucopenic index test.

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THE VARIATIONS IN THE LEUCOCYTE COUNT IN RELATION TO THE LEUCOPENIC INDEX*†

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THE leucopenic index was introduced in 1934 by Vaughan[1] as a test of food allergy. It was based on the astute observation that the colloidoclastic crisis test of liver function proposed by Widal, Abrami, and Iancovesco[2] was in reality a leucopenic response to milk by allergic individuals.

Since the value of the test depends directly upon the accuracy of the leucocyte count, it seemed desirable to reinvestigate the subject of leucocyte counting with particular reference to the leucopenic index. Vaughan[3] and most later workers have utilized a drop of not less than 1,000 cells as their criterion of a positive test. For this reason, the accuracy of the individual counts must be such that variations from all causes other than allergy are smaller than this figure. Accordingly, the purpose of this investigation is to study the factors which influence the white cell count; to determine their magnitude; and to suggest, if possible, practical means of eliminating variations sufficiently large to give false positive or false negative leucopenic indices.

The sources of leucocytic variation and inaccuracy of counts may be grouped under three headings: physiologic changes, technical errors, and the element of chance.

Physiologic variations consist of leucocyte changes due to the effects of redistribution of white cells, vascular changes, and digestion of foods. Redistribution is caused principally by postural changes and exercise according to Garrey,[4] and Garrey and Butler,[5] and may alter the count by many thousand cells. Shaw[6] pointed out that the leucocyte count was much more variable with vasoconstriction and tended to be higher than with vasodilatation. There is little agreement in regard to the effect of food. Garrey and Butler[5] and Raisky[7] deny the existence of digestive leucocytosis and explain previous results on the basis of postural change. Lawrence, Stephens, and Jones,[8] however, believe that a moderate digestive leucocytosis occurs. Vaughan[9] intimates that these contradictory findings may result, in part, from failure to consider the factor of food allergy. In doing the leucopenic index

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these physiologic variations can be minimized by performing the test on the fasting patient at complete rest, and letting at least two drops of blood escape to permit vasodilatation to set in.

Technical errors can be kept at a minimum with care, so that they account for a negligible fraction of the total variation. Only three points need be considered here. First, when the proper column of blood is secured in the stem of the pipette, the tip is wiped of excess blood by nonabsorbent material and the blood column is drawn slightly up the tube to prevent loss when pipette is first dipped in diluent (Nicholson).[10] Second, suction must be continuous until dilution is completed. Simultaneous rotary agitation must be made during part of this time to prevent clumping of cells. Third, the counting chamber must be filled rapidly and smoothly. For a detailed description of this technique see Osgood.[11]

The element of chance undoubtedly plays the most important part in the blood count and requires a statistical approach for its evaluation. Ponder, Saslow, and Schweizer[12] showed that counting an insufficient number of cells resulted in wide variations and gave conclusive evidence that the apparent physiologic rhythms of Sabin[13] and coworkers, resulted from counting only 4 mm. squares (about 200 cells).* Ponder[12] and associates showed variations in counts up to 2,640 from the same specimen when counting 200 cells. This dropped to 600 when 800 leucocytes were counted (about 16 squares).

Bryan and Garrey[14] brought out the importance of proper shaking of the pipette to make an even distribution of cells in the diluting fluid, and designed a rotary mechanical shaker to accomplish this. Another factor which might equally well be considered under technique is the effect of drying of fluid in the counting chamber during or before counting. When drying occurs at the corners of the chamber, large numbers of cells collect and are swept onto the ruled area by the receding meniscus. The number of cells is changed at distances of up to 1 mm. from dry areas. This difficulty may be obviated by rapid counting, by humidification, or when more than one chamber is counted, it may be kept under a Petri dish lined with moist blotting paper. This can also be utilized during the three-minute period allowed for settling of cells in the counting chamber.

Our first experiment consisted of serial counts at fifteen-minute intervals made upon young, normal adults (8 females, 6 males). There were 17 counts in each series. The subject ate no breakfast and rested in bed one hour before the first test. The counts were made with care, using routine technique of hand shaking three minutes, and counting 8 squares, 4 on each side of chamber. The same pipette, chamber, and

*All squares referred to in this paper consist of an area of 1 sq. mm.

cover glass were used for a series, and the cover glass applied the same way to the chamber. The results showed apparent rhythms with wide variations. In each series there was at least one point where the difference between successive counts was 1,000 or more, while the maximum difference between any two counts of a series was 1,500. The maximum variation between adjacent counts was 2,500 cells. This experiment apparently demonstrated rhythms similar to those found by Sabin[13] and associates, although 8 mm. squares were counted per test. These variations were later shown to be a combination of physiologic changes and an inadequate technique. Leucopenic indices run under similar circumstances would have been of very questionable value. It was considered necessary to repeat this type of experiment with improved technique, principally, improved mixing of cells and counting a larger area. Before doing this, a series of experiments were performed to eliminate inaccuracies of technique and to give some basis of comparison with results in the literature.

TABLE I

MILLIMETER SQUARES

	STANDARD DEVIATION OF CELLS PER SQ. MM.	NO. MM. SQUARES COUNTED	MEAN NO. CELLS PER SQ. MM.	COEFFICIENT VARIATION	PROBABLE ERROR	TIME OF SHAKING MINUTES
Hand shaken	± 6.37	80	67.6	± 9.4	0.48	Three
Rotor shaken	± 5.18	80	74.8	± 6.9	0.39	Three

TOTAL COUNTS

	MEAN COUNT	STANDARD DEVIATION OF COUNTS	COEFFICIENT VARIATION	PROBABLE ERROR	TIME OF SHAKING MINUTES
Hand shaken	13,350	± 407	± 3.05	86.5	Three
Rotor shaken	14,900	± 550	± 3.6	117	Three

First, a comparison was made of distribution of cells in the counting chamber after shaking by hand and shaking an equal length of time in the Bryan-Garrey rotor. In this experiment 10 counts were taken from the same pipette with hand shaking. The same pipette was refilled and similar counts repeated after shaking in the rotor. The statistical analysis of the counts is shown in Table I.

This experiment was repeated trying one minute, three minutes, and six minutes shaking time with the results as indicated in Table II.

These results show no significant difference between the two methods when the time is three minutes or over. The hand shaking consisted of a vertical excursion of about one foot, holding the pipette between the thumb and forefinger, repeated about 120 times per minute. The second finger moving farther than the thumb imparts a rotary as well as a vertical motion. The standard deviations obtained are practi-

TABLE II

MILLIMETER SQUARES

	NO. MM. SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	TIME MINUTES
Hand shaken	80	43.4	± 4.64	± 10.7	0.07	One
Rotor shaken	80	49.7	± 8.47	± 16.9	0.63	One
Hand shaken	80	40.1	± 4.4	± 10.9	0.33	Three
Rotor shaken	80	38.3	± 5.4	± 14.1	0.407	Three
Hand shaken	80	38.8	± 4.3	± 10.7	0.31	Six
Rotor shaken	80	43.8	± 4.9	± 11.4	0.37	Six

TOTAL COUNTS

	MEAN COUNT	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	TIME MINUTES
Hand shaken	8500	± 403	± 4.7	86	One
Rotor shaken	9600	± 1220	± 12.6	260	One
Hand shaken	7940	± 310	± 3.9	65.9	Three
Rotor shaken	7650	± 538	± 7.03	114	Three
Hand shaken	7550	± 458	± 6.06	97.4	Six
Rotor shaken	8500	± 387	± 4.5	82.3	Six

cally the same as those obtained by Bryan, Chastain, and Garrey.[15] The rotary shaker was used in succeeding experiments.

We next investigated the evenness of distribution in the regular glass counting chamber and the metal surfaced glass type (Spencer Brightline), since the latter appears to fill more easily. The results indicate slight superiority in evenness of distribution on the part of the glass-metal chamber. A definite reduction of fatigue was also noted (Table III).

TABLE III

MILLIMETER SQUARES

	NO. MM. SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	TIME MINUTES
Brightline	80	45.31	± 5.69	±12.3	0.429	Three
Glass	80	38.31	± 7.35	± 19.8	0.554	Three

TOTAL COUNTS

	MEAN COUNT	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	TIME MINUTES
Brightline	9000	± 750	± 8.33	160	Three
Glass	7500	± 866	± 11.0	181.1	Three

This comparison was repeated with a larger series of counts with similar results as shown in Table IV.

These findings consistently favor the metal surface chamber for evenness of distribution and, as expected, also show a smaller variation between total counts. The Spencer Brightline counting chambers were used in all other experiments.

TABLE IV

MILLIMETER SQUARES

	NO. MM. SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR
Brightline	160	±45.8	±4.97	±10.85	0.265
Glass	160	±45.4	±6.78	±14.9	0.36

TOTAL COUNTS

	NUMBER COUNTS	SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	RANGE BETWEEN HIGHEST AND LOWEST COUNTS
Brightline	10	16	9080	± 366	± 4.04	78.5	1300
Glass	10	16	9100	±1021	±11.22	217.6	3050

Two investigations were made concerning the filling of the counting chamber. The first compared slow but exact filling of chamber with more rapid smoother filling. Two chambers were filled alternately from same pipette (Table V).

TABLE V

MILLIMETER SQUARES

	NO. MM. SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR
Rapid smooth filling	80	41.5	± 5.75	±13.8	0.43
Slow, jerky but exact filling	80	42.0	±10.62	±25.1	0.8

TOTAL COUNTS

	MEAN	SQUARES COUNTED	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR
Rapid filling	8250	8	± 774	± 9.38	165
Slow filling	8350	8	±1624	±19.6	346

Although the number of counts is relatively small the above comparison emphasized the importance of a smooth rapid filling of the chamber. Since rapid filling tends at times to a slight overfilling so that there is a small meniscus overhanging the edge, the next comparison was made between deliberate overfilling and as exact filling as possible made at the same speed (Table VI).

TABLE VI

	NO. MM. SQUARES COUNTED	MEAN	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR
Rapid accurate filling	24	47.8	±6.11	±12.8	0.84
Rapid slight overflowing	20	52.7	±7.6	±14.5	1.15

While this comparison suggests that a slight overfill causes no appreciable difference, a much larger number of squares need be

counted for proof. The technique of Osgood[11] was followed in the actual filling of the counting chamber.

The final experiment on leucocyte counting utilized every possible precaution and was designed to test the accuracy of our technique before doing leucopenic indices. Four pipettes were used to collect blood from the same wound, as nearly simultaneously as possible. These pipettes were calibrated by the method of Dunn[16] and the following negligible errors found: 0.22 per cent; 0.5 per cent; 0 per cent; and 1.5 per cent. After dilution, the pipettes were placed in the rotor, which was run continuously until the experiment was completed. A total of 576 millimeter squares was counted, and the total leucocyte counts calculated on a basis of 8, 16, and 36 squares per count. The results are shown in Table VII.

TABLE VII

MILLIMETER SQUARES

NO. SQ. MM. AREAS COUNTED	MEAN NO. CELLS PER SQ. MM.	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR
576	36	±4.54	±12.6	0.127

TOTAL COUNTS

NO. SQUARES COUNTED PER COUNT	NO. COUNTS	MEAN TOTAL LEUCOCYTE COUNT	STANDARD DEVIATION	COEFFICIENT VARIATION	PROBABLE ERROR	GREATEST DIFFERENCE BETWEEN ANY TWO COUNTS
8	72	7070	±433	±6.15	34.5	1800
16	36	7072	±353	±4.99	39.49	1350
32	18	7059	±273	±3.94	43.49	900

A possible pitfall in this type of experiment is the tendency of leucocytes to adhere to the wall of the glass pipette. As a result the last few counts tend to be low when the fluid escapes from the pipette by gravity as usual. However, if the last drop is forcibly ejected by blowing, the adherent leucocytes are dislodged and a very high count is obtained. Consequently, we did not count the last few drops in each pipette. It is also possible that this type of experiment, for the reasons just given, may introduce some additional error not involved in ordinary blood counts.

The results of this experiment indicate that it was necessary to count 32 squares per count to be certain that the greatest variation between any two counts due to chance alone was less than 1,000 cells. However, by counting 16 squares, a few counts had a variation of more than 1,000 cells between them (maximum 1,350). Nevertheless, no count differed from the mean by as much as 1,000 (maximum 725). The evenness of leucocyte distribution and variation of our total counts as shown in the statistical analysis compared favorably with the counts of other investigators. This conforms to Pearl's[17] statement that roughly three times the standard deviation on either side of

the mean will include all the variates. Fig. 1 illustrates this relationship of the standard deviation to the various counts when 32 squares are observed, and when 16 squares are used per count. It is apparent from these figures that leucocyte enumerations made with 16 squares per count would scarcely be accurate enough for investigation but would probably serve as a minimum standard for clinical use. Denny[18] has already recommended such a minimum and was the first to report extensive clinical studies of the leucopenic index with counts analyzed by statistical methods.

In this connection, the authors wish to point out that the standard deviation is preferable to the coefficient of variation for the purposes of comparison when a definite numerical criterion (1,000) is used and similar data compared.

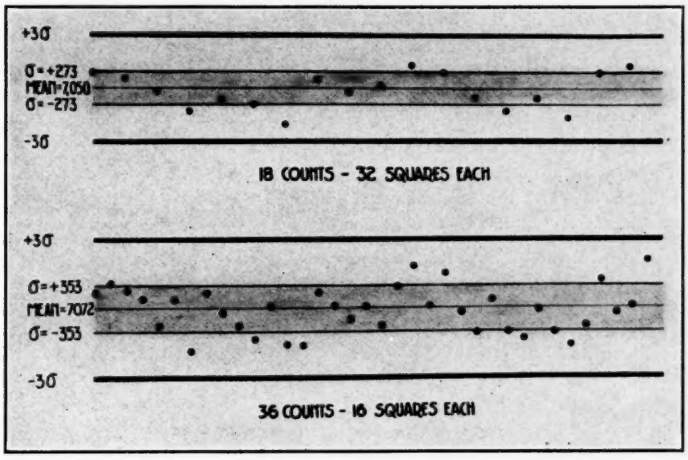

Fig. 1.

Having demonstrated that technical and chance variations could be reduced to the point where a thousand cell criterion would always be significant, a study of normal individuals was undertaken. For each count 32 squares were observed and exactly the same technique followed throughout. Fig. 2 shows the results of a protracted series of counts on resting and fasting normal adults. Since the distribution of cells in these counts was shown to be as even as in the preceding experiment, the definite variations shown were considered to be real. Most marked changes were observed in the case of S. I. This subject developed a violent headache, showed flushing of the face, and was acutely uncomfortable throughout most of the experiment. Case M. C. received disturbing news at the start of the series, but was reassured and slept through the last 6 counts. The other two cases remained within the 1,000 cell range.

The wide fluctuations in the first two counts were apparently true physiologic redistributions, and illustrate the difficulty of eliminating

the causes of this type of variation, even when precautions are observed in regard to rest, food, and vascular changes. The remarkably slight variations observed during sleep further confirm the idea that physiologic changes account for the wider fluctuations in the series. Accordingly, a second series of normal individuals was investigated under conditions of rest and quiet with no emotional disturbances.

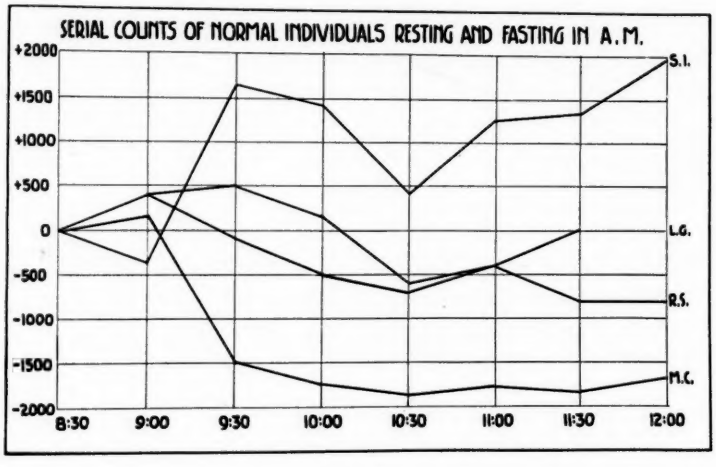

Fig. 2.

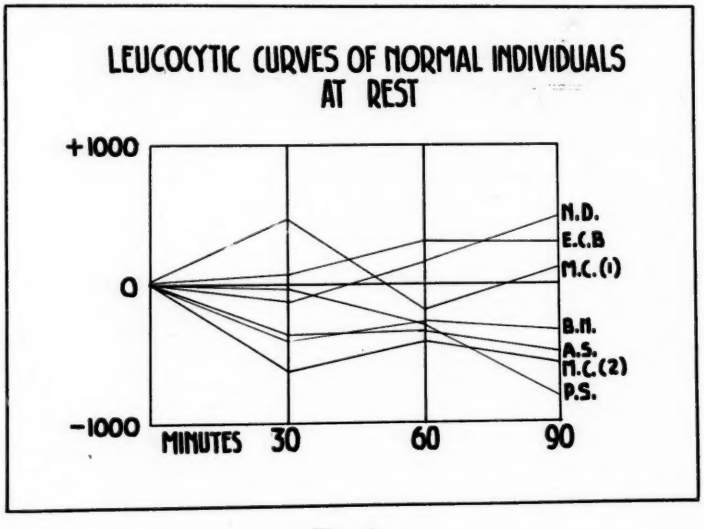

Fig. 3.

These tests were run in the same manner as leucopenic indices, except that 3 were performed in the afternoon and one in the evening. Only one-half hour of preliminary rest was allowed. Fig. 3, representing the results of this experiment, shows scarcely more variation in the counts than would be expected from chance. Only one curve

(Case P. S.) showed a consistent drop, although it did not fall 1,000 cells. On questioning, it was found that the individual had eaten dinner about one hour previously and admitted that on occasions she had slight nasal congestion after meals. It is quite probable, there-

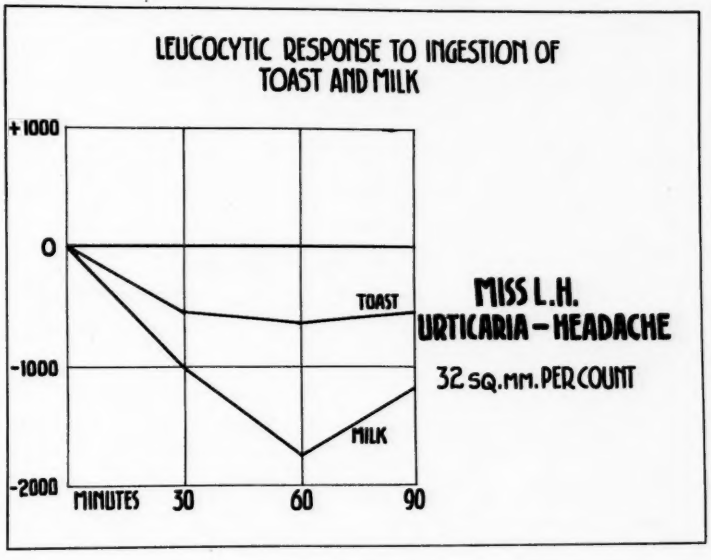

Fig. 4.

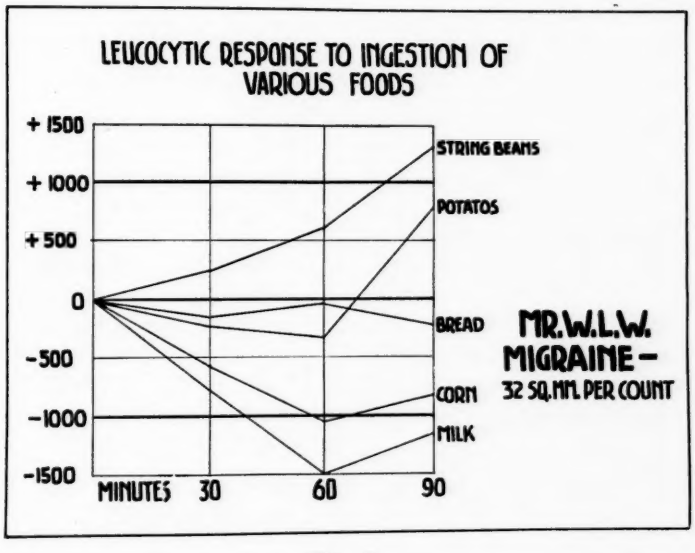

Fig. 5.

fore, that this one curve represents an actual leucopenic response to some food. Figs. 4, 5, and 6 represent leucopenic indices run on individuals with known allergic symptoms. All positive counts in these figures were due to foods known to be causative of symptoms. In

each case the usual critical technique was employed with 32 squares per count being observed. In addition, the patients were fed so as to avoid the necessity of change in posture or excessive movement. The

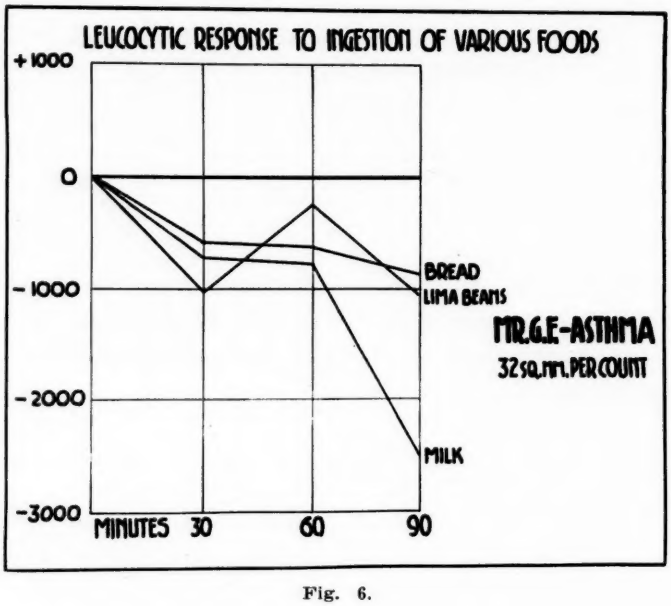

Fig. 6.

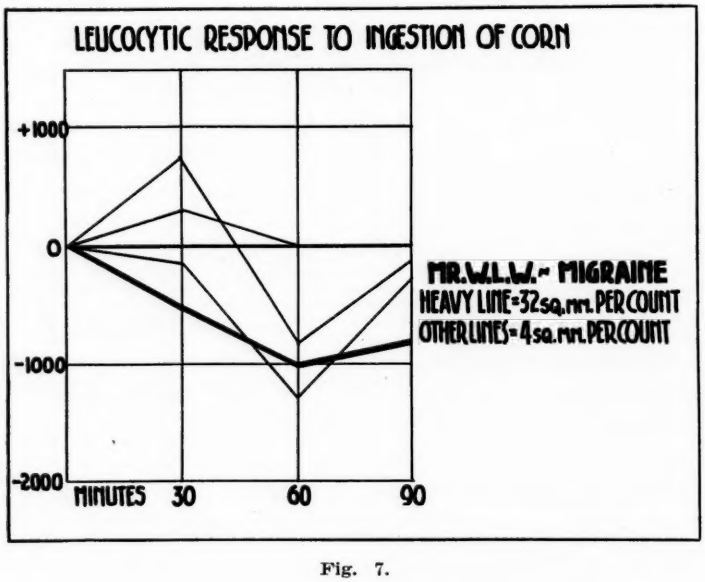

Fig. 7.

only curves showing a suggestion of the Z shape (Fig. 6) was in a child, age 8 years, who was restless during the test.

Fig. 7 was particularly interesting, since it represented the result that can be expected from counting an inadequate number of cells.

The heavy line shows the probable true curve based on 32 sq. mm. per count. The lighter lines show the type of curve obtained when the counts are based on only 4 squares chosen at random from the 32 squares, for example, the first 4 squares, the last 4 squares, etc.

Blood smears were taken simultaneously with each count made for the leucopenic indices. Differential counts were made from these smears, 500 cells being studied in each instance. While some of the individuals showing positive responses to food, also had eosinophile percentages above normal, in no instance was there a coincident significant fluctuation during the period of the tests.

The acetone counting fluid of Squier and Madison[19] for estimation of eosinophiles during the leucocyte count was tried. The differential count was preferred because the acetone fluid was not satisfactory for the leucocytes, although eosinophiles stained very well.

DISCUSSION

Our findings indicate that several factors may cause variations in excess of 1,000 leucocytes in the blood of normal individuals when serial counts are performed. These factors are physiologic redistribution, inadequate sampling, that is, counting too few cells, and a summation of technical errors.

Physiologic changes can be reduced in most cases to relative insignificance by rest, elimination of fear and other emotional disturbances, and the maintenance of the same posture. The fasting state is of course necessary when a leucocyte index is to be run. This precaution is taken in order to avoid conflicting results.

Inadequate sampling is probably the chief source of erratic curves and apparent wide variations. Counting 32 sq. mm. will give an adequate sample with a safe margin. This number of squares can be counted readily if the interval between counts is increased to one-half hour. Counts based on 16 squares may suffice in clinical usage but leave no margin of safety, particularly in borderline cases.

Ordinary technical errors need not be considered with careful experienced workers. However, either a mechanical rotor, such as the Bryan and Garrey type, or rigidly controlled hand shaking must be employed to assure even distribution of cells. An exacting technique must also be followed in filling the counting chamber.

By following the above technique, standard deviations of total counts can be held to less than one-third of the thousand cell criterion of Vaughan. We believe that this degree of accuracy will give relatively smooth curves of leucocytic response, thus eliminating widely fluctuating leucopenic index curves, particularly those which lie deep in both the positive and negative ranges. Coincidentally there should be fewer borderline tests, and consequently a higher correlation of posi-

tive tests with symptoms of food allergy. It is hoped that this critical hematologic technique in the hands of competent clinical allergists will result in a more exact evaluation of the leucopenic index.

CONCLUSIONS

1. The sources of variation of the leucocyte count with reference to the leucopenic index technique are reported.

2. Means of reducing these variations to a minimum are suggested.

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Society Proceedings

THE ASSOCIATION FOR THE STUDY OF ALLERGY

ATLANTIC CITY, N. J., JUNE 7, 1937

The Leucopenic Index and Differential Counts in a Case of Gastric Ulcer. RALPH H. SPANGLER, M.D., Philadelphia, Pa. (Abstract.)

THE reports of Vaughan[1] and Rinkel[2] in 1934, 1935, and 1936 on "The Leucopenic Index in Allergy," and L. P. Gay's[3] report on "The Leukopenic Index as a Method of Specific Diagnosis of Allergens Causing Peptic Ulcer," together with the request contained in the Editorial of the J. A. M. A. on June 6, 1936, suggesting detailed counts as an aid in evaluating the leucopenic index in cases of food allergy, led to an investigation of the leucocyte response to specific foods in a patient who presented clinical symptoms of peptic ulcer, confirmed by x-ray. Such a study seemed to be of especial interest when I recalled a case, previously reported,[4] of an asthmatic patient whose sensitivity to pork appeared to illustrate well the so-called "hemoclasic crisis of Widal."

CASE REPORT

The patient, a tailor, was a white male, single, aged 27 years, height 5 feet 7 inches. His weight was 120 pounds when first seen, April 14, 1936, having weighed 137 pounds when 21 years of age.

Chief Complaint.—Patient had given up work six weeks previously and had become very neurotic over loss of weight and vague upper abdominal distress.

Allergic History.—The personal allergic history of this patient was negative, except perhaps for the fact that he had "spasms" when two months of age. In the immediate family history, however, his father had asthma and died at 40 years from ulcerative colitis, diagnosed at autopsy. The patient's mother suffered with periodic headaches until menopause, and his 25-year-old sister has migraine. His maternal grandmother and a maternal uncle have asthma.

Physical Examination and Laboratory Findings, including Wassermann tests, were practically negative.

X-ray Report by Dr. E. B. Spachman revealed, "Ulcer on lesser curvature of stomach."

Cutaneous *Food Tests* by scratch method were negative for milk, egg, wheat, cocoa, and cheese, the chief foods patient had been eating the previous month.

Leucocytic Reaction Tests to Food.—Milk reacted with a leucopenia, a 4,000 leucocyte drop, and a left shift, with 34 per cent nonfilamented (young) neutrophiles. (Normal range of nonfilamented 8 to 16 per cent.[5])

Egg and wheat were followed by a leucocyte rise of 1,800, no leucopenia and no left shift, with nonfilamented neutrophiles of 9 per cent.

Then for twenty-six days on a soft, nonroughage diet minus milk, the patient reported he was in fine shape until after a "food spree" (ice cream, peanuts, and beer) on twenty-seventh day when severe upper abdominal pain occurred. Eighteen

hours after the "food spree" the white count was 4,800 and there was a slight left neutrophile shift (18 per cent nonfilamented forms). After seven days again on a soft, smooth diet minus milk, a "milk-leucocytic reaction" test showed a leucopenia drop of 2,600, and a left neutrophile shift to 33 per cent.

Oral Desensitization to Milk.—The patient, however, was losing weight (5 pounds in five weeks) and owing to his neurotic attitude, in spite of the absence of pain, but believing that no milk caused the decline in weight, he readily agreed to the suggestion that an attempt be made to orally desensitize him to milk. Accordingly, the patient abstained from all food except milk, and took 3 oz. of milk and 1 oz. of cream every hour for thirteen hours of the twenty-four (about 2,000 calories a day) over a period of seven days and then for a second week one egg per day was added to the hourly milk and cream feeding. This is practically a first two-week Sippy regime except that the patient was not put to bed.

There was no pain and no neutrophile left shift after the first week. The patient was kept on the hourly milk and cream feedings for ten weeks with soft foods added gradually. His weight was regained and with the clinical symptoms absent he appeared to have been orally desensitized to milk. He returned to his regular work at the end of four months, and by the end of six months was eating a general mixed diet which included a quart of milk daily.

Leucocyte Counts During and After Milk Desensitization.—Over a period of three months total white and differential counts were tabulated once a week and four milk-leucocytic reaction tests were made. The degree of leucopenia and the neutrophilic left shift gradually decreased during the ten-week period of hourly milk and cream feedings. A milk-leucocytic reaction test, made three months after starting the oral milk desensitization, showed a leucocytic rise of 600 and no left shift. Four months later, there was a leucocyte rise of 3,500 and no left shift after a milk-leucocytic reaction test.

One year after the leucopenia to milk was originally determined in this patient (May 9, 1937), a milk-leucocytic reaction test showed a leucocyte rise of 2,800 and no neutrophile left shift.

Clinical Results.—One year after the diagnosis of the gastric ulcer the patient continued free from abdominal distress and was eating a general mixed diet, including a quart of milk daily. He worked at his trade regularly for eight consecutive months and maintained a weight of 133 to 135 pounds. Three milk-leucocyte reaction tests made during the last six months after he returned to work all responded with a total leucocyte rise and no left neutrophile shift.

On May 8, 1937, Dr. Spachman reported as follows: "The x-ray check-up study shows no defect at this time. This would indicate complete healing of the previously described ulcer on the lesser curvature of the stomach reported May 6, 1936."

On May 29, 1937, a fractional gastric analysis with an Ewald test meal showed the combined acids 70° and free HCl 50°. No blood or mucus was present.

COMMENT

Without taking into consideration the question of a fasting or digestive leucocytosis or leucopenia, or the possible part played by chemical neutralization of gastric acidity, the findings in this case of gastric ulcer (confirmed by x-ray) indicate: (1) that Vaughan's leukopenic index is of value in the diagnosis and dietary management of gastric ulcer, and (2) that the differential leukocyte count done by the Farley (filamented-nonfilamented) modification of Schilling's neutrophile shift, serves as a check-up of the leucopenic index; and further may offer in itself a more simple, less time-consuming procedure. This, possibly, may

likewise be a more accurate method of indicating food sensitivity in gastrointestinal allergy, than the more or less variable skin test methods. Moreover, the neutrophile shift in the differential count offers considerably less chance of error than does the total white count technique, with its variation in pipette dilution and in the frequent unequal distribution of the cells in the counting chamber. Finally the clinical results in the case, including freedom from pain, weight regained, the patient's return to regular work and on a general mixed diet, including milk, in four months' time, together with the x-ray findings showing no evidence of gastric ulcer, suggest that a possible mechanism underlying the Sippy and similar regimes used in the treatment of peptic ulcer may be one of oral desensitization to milk.

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DISCUSSION

DR. WARREN T. VAUGHAN, Richmond.—The leucopenic index has been subjected to two criticisms, first, that you cannot make a satisfactory or accurate white count within the limits prescribed in the index; and, second, the question is raised whether one really does get a leucocyte fall following contact with an allergenic food. In an editorial in the J. A. M. A. last year, a number of authors were quoted to show that normally, even fasting, you can get such a wide variation in leucocyte counts, that the method cannot be satisfactorily used. These were all excellent articles, but I suspect that the man who wrote the editorial paid more attention to the summary and conclusions than he did to the articles because on analyzing them, I found that for the serial counts done in the early hours of the morning, up to twelve o'clock, the range was within 2,000 in 80 odd per cent, which is what we claim for the method; 1,000 up or down. Provided the counting is done between nine and twelve in the morning, the method is accurate and is indeed confirmed actually by the papers quoted in the editorial.

We ourselves do two fasting counts and we find that in a series of 500 leucopenic indices, the fasting counts showed no more variation than 1,000 in 96 per cent and 99 per cent showed no more variation than 2,000. That is 1,000 up or down. Only 1 per cent varied more than 2,000.

With regard to the question whether there is a leucopenia in the allergic response to foods—that was demonstrated long ago by the French who have done a lot of work on it. The difference is that they studied an explosive case as a rule, and studied the white count as an interesting observation in the single case. What we have done is to take that phenomenon and apply it as a diagnostic measure in unknown sensitizations. We have reversed their procedure. Really the only question is whether or not a drop of 1,000, which is purely arbitrary, should be changed to require a greater drop. With a drop of 1,000, which is, as I say, arbitrary, but also based upon my own experience, one will get some false positive reactions, but if one requires a drop of 2,000, one will miss many more true positives. So that with a drop of 1,000, you will get some false reactions, but you will catch more of the true positive reactions. I feel from my own experience that a drop of 1,000 is about right.

We have found that the original white count level plays a part. When the fasting count ranged from 2,500 to 4,500, only 2.6 per cent varied more than 1,000. When the original white count was over 10,500, 30 per cent varied more than 1,000. Therefore, one should be cautious in interpreting a curve with leucocytosis. One should require more of a drop certainly than 1,000.

The advantages of the method, as I see it, are first, one can test with things that cannot be used in a skin test. You can test with drugs. The response to drugs is the same as the response to foods. You can test with food combinations. The patient suspects a particular dish and you can test this combination. You can test with things that are of unknown content, such as Ovaltine and proprietary preparations, or things that are difficult to get for skin testing, as Coca-Cola.

There is one very distinct advantage. Dr. Rackemann said years ago that a positive skin reaction was an historic landmark, that it indicated present trouble or past trouble, or potential future trouble. The leucopenic index, in my experience, indicates present trouble. As soon as the active symptoms from sensitization to the food have disappeared, the leucopenic index will become negative in the majority of cases. It is a more reliable index of actual present trouble and does not give you as many false positives due to the historical factor.

Gay and Rinkel suggested that when there is a question concerning a food, and if the leucopenic index is negative, one should feed that food for several days and then repeat the leucopenic index; it will become more strongly positive. I have myself felt that it ought to do the reverse, but we have found that it is true, if a person continues to eat a food for some time, the leucopenic index is likely to become more strongly positive, which is the reverse of the experience of Dr. Spangler, although in his last count there was a borderline drop.

Dr. Squier, I feel, has added very definitely to the value of the leucopenic index with his eosinophile count, because it provides a double check.

Now just two slides which I will show you very quickly.

(Slide.) This is a patient who had skin tests and then leucopenic indices with 25 different foods. There was a record made of whether she did or did not have symptoms after eating the food. The symptoms and the leucopenic index correspond 14 times; the symptoms and the

skin tests only correspond 9 times. The leucopenic index failed to correspond only 6 times, and the skin test failed to correspond a total of 15 times.

(Slide.) This represents 41 tests done in the same way. The symptoms and the leucopenic index corresponded 28 times as contrasted with only 12 times with skin tests. Here, again, there are only four times that the leucopenic index failed to correspond against 12 for the skin tests.

DR. BRET RATNER, New York.—Does Dr. Vaughan believe that what produces a drop in white cells can at the same time produce an increased shift to the left, which indicates immature cells coming out of the bone marrow? It seems rather an amazing situation that you can have a drop in the total count and at the same time an increase in immature cells. During a leucopenia there is a destruction of cells, and it is only when the polys increase that immature forms can appear.

DR. VAUGHAN.—I do not know; we have not done differential counts.

DR. LOUIS ELWOOD PRICKMAN, Rochester, Minn.—For some time I have been much interested in the leucopenic index test as described by Dr. Vaughan, and I represent what might be termed the "loyal opposition."

I have tried to keep an open mind about the leucopenic index, and really do not oppose its use, but there are several things about the test with which I cannot quite agree. Let me first confess that I agree with the views of the editorial to which Dr. Vaughan has just referred in his discussion of this paper. I am sure this is no news to Dr. Vaughan, because I discussed the matter with him before the editorial appeared and explained my views and the data I had at the time. Dr. Vaughan deserves and has received a great deal of credit for bringing out this test, and I believe the principle underlying the test is deserving of more study. My first criticism of the test arose from the fact that Dr. Gay, in his report, said that foods were withdrawn from patients' diets because the use of those foods was followed by a drop in the leucocyte count of 200, 400, 600, 800, 1,000, or 2,000 cells, which as near as I can tell from a few preliminary studies of my own, and from the literature reviewed in part in the editorial, are probably not significant variations.

Another matter which concerned me was the lack of published controls for the leucopenic index test. Before I talked over the test with Dr. Vaughan, we, at the clinic, performed a number of tests in the morning on normal, fasting individuals. We first obtained the mean leucocyte level of each individual and then, without giving the individual food, we performed subsequent leucocyte counts according to the method of Vaughan, and frequently obtained very definite and sometimes marked leucopenias, or "incompatible" curves according to Rinkel's terminology. Therefore, if under the controlled conditions such as obtained when these tests were performed, a positive leucopenic index can be demonstrated in work with normal individuals who have not had food, what conclusions can be drawn from similar leucopenias which appear after eating test foods? The explanation for these discrepancies lies, I believe, in the errors or inaccuracies inherent in the technique of obtaining blood and counting the leucocytes. The exact error is not as yet known as we should know it but when considered statistically, the error is great. It has been shown in one study that there is an error of plus or minus 8

per cent, making a possible variation of 16 per cent in counting leucocytes under ideal circumstances, circumstances which are greatly different from those under which the ordinary leucopenic index test is performed, so that in practice the error in counting leucocytes is undoubtedly a great deal more than 16 per cent.

The case reported today by Dr. Spangler interested me too for several reasons. In the first place, the patient, who had a large gastric ulcer, was obviously ill and had an abnormal condition of his blood to start with. He had, for instance, a definite anemia, there was very little more than 3,000,000 erythrocytes and also the earliest count of nonfilamented cells was more than the usual upper limit of normal; this was before the reactions of this count to the ingestion of certain foods were studied. Just to be a little bit contrary, I should like to explain the changes which Dr. Spangler noted in the count of nonfilamented cells as variations due to the infection present; in this case, in the gastric ulcer. Such variations in the condition of the ulcer would seem to me just as reasonable an explanation for the changes Dr. Spangler noted, as to explain them as due to changes made in the patient's diet. Lastly, in so far as the observations in this case of the leucocytes and the leucopenic index are concerned, from my own experience, I would not consider the variations in the leucocyte counts much, if any, beyond what the limits of error of the test would adequately explain.

Dr. Theodore L. Squier, Milwaukee.—I feel that one of the principal objections that has been raised to the leucopenic index is that it is too time-consuming and allergists do not wish to devote the additional time for study that this test requires. Certainly it is true that those who have used it and used it consistently are increasingly convinced that it gives clinical information of value.

When I first began to use the leucopenic index, I used it solely in studying some blood conditions, particularly thrombocytopenic purpura, and in so doing became more and more convinced of its clinical value in food allergy.

As Dr. Vaughan has pointed out, I too have never seen a clinical response to a food at a time when the leucopenic index was negative—that is, when normal leucocytosis occurred after ingestion. I have seen, as Dr. Vaughan has also mentioned, a negative leucopenic index to a food that later, after the food had been eaten freely, gave a positive leucopenic drop and at that time produced clinical symptoms. So there is a variation in clinical tolerance. Frequently I have seen positive skin tests to foods which gave negative leucopenic indices on ingestion and in each instance there has been no indication whatsoever of intolerance to that food.

I feel that the eosinophile count made simultaneously with the leucocyte count is distinctly of value. In the first place, it is a simple thing to do. It does not increase the time involved by more than a moment or two when the counts are made simultaneously in the counting chamber. It does give distinct information of especial value in the so-called indeterminate test where there is a fall of less than 1,000 leucocytes. In such instances where there is a distinct eosinophile rise, usually occurring in thirty minutes, I feel that food intolerance is much more probable than when there is an indeterminate leucocyte fall without a rise in eosinophiles.

DR. SPANGLER (closing).—The points brought up are all very interesting and apropos. Dr. Vaughan advises that counts should be made in the morning on an empty stomach. It has been my understanding from Dr. Vaughan's reports that the importance of making the counts in the morning was so that the first count could be made on an empty stomach and when the patient has had little or no exercise. I would like to ask Dr. Vaughan if the hour of the day matters provided the patient has had no food for ten or twelve hours, and has avoided exercise.

Dr. Ratner's question, I take it, is the crux of the blood findings in this case report. The drop in the total leucocyte count with an accompanying left shift would seem an unusual or irregular reaction. But as Dr. Prickman has stated, the activity of the ulcer may explain the left shift, and I would add that the ulcer's activity may possibly be induced by sensitivity to the food ingested. In this particular case it was milk. The drop in the total white count, i.e., the leucopenia following the ingestion of a specific allergen is what Vaughan, Rinkel, Gay, and others have pointed out as a characteristic leucocytic response in allergy. Judging by the differential counts made in this case, the left shift may also be a characteristic leucocyte response and occurring at the same time as the leucopenia the left shift may possibly also be regarded as a factor in the leucocytic mechanism of the allergic reaction.

Dr. Prickman, as he has previously done, raises very interesting technical and physiologic questions which I will not attempt to discuss, important as they undoubtedly are.

Dr. Squier's interesting report of a year ago in which he found a correlation between the leucopenic index and an eosinophilia did not seem to obtain in the present case. We made a total of 55 differential counts on this one patient. Three of them show 5 per cent of eosinophiles; four 6 per cent, and four 7 per cent. Even these low percentages and infrequent occurrences of an eosinophile increase did not seem to bear a definite relation to the ingestion of the presumably incriminating allergen, milk.

From the clinical standpoint, I just want to add in conclusion that "the proof of the pudding is in the eating." The patient is well, has regained his weight, eats a general mixed diet including milk, and the x-ray films made a month ago show that no ulcer is now present.

NOTICE OF MEETING

The Annual Meeting of the Association for the Study of Allergy will be held at the Hotel Empire (formerly William Taylor Hotel), San Francisco, June 10 and 11, 1938.

THE SOCIETY FOR THE STUDY OF ASTHMA AND ALLIED CONDITIONS

ATLANTIC CITY, N. J., MAY 1, 1937

Active Sensitization of Human Uterine Muscle. *Preliminary Report of Experimental Attempts.* LOUIS TUFT, M.D., Philadelphia, Pa. (Abstract.)

IT IS generally believed by immunologists that the pathology of human allergy does not include smooth muscle sensitization. In this respect it is believed to differ from the pathology of animal anaphylaxis. Nevertheless, the literature contains references indicating that sensitization of the muscle structure of the uterus, bladder, or bronchi does occur and accounts for clinical symptoms localized to these structures. For example, Duke and Rowe refer to dysmenorrhea and to bladder symptoms as a result of food allergy; there are also occasional references of this possibility in discussing the pathology of asthma. These beliefs would seem to be based upon clinical rather than experimental evidence, obtained in the human being.

In order to obtain more satisfactory criteria either for or against this assumption, a series of experiments were planned to determine whether active sensitization of smooth muscle could be induced in the human being. The general plan of procedure was to inject individuals about to be operated on for a uterine disorder with immune serum prepared from horses, to allow an interval to elapse sufficient for antibodies to form and at that time operate and obtain a strip of uterine muscle. This strip was then to be tested for sensitization to horse serum by the Schultz-Dale technique. The present report is a preliminary report of the results of experiments carried out up to the present time.

The first attempts were made with ordinary uteri obtained in gynecologic operations. It was soon found, however, that such uteri were usually fibrotic and did not adapt themselves to this type of experiment. It was then decided that the muscle of the pregnant uterus, being well developed, would lend itself ideally for experimental purposes.

Patients were chosen, therefore, who were to have elective cesarean sections performed upon them. These patients were tested for sensitization; if absent and whenever possible they were given 50 c.c. antistreptococcic serum intravenously. Symptoms of serum sickness were watched for and skin tests and antibody determination made on such patients. When evidence of sensitization was present and antibodies found present in the blood stream, operation was then performed and a strip of uterine

muscle obtained from the incision in the uterus. This was immediately placed in Ringer's solution and tested for sensitization in the Schultz-Dale bath.

In order to determine the technical efficacy of our method, experiments were carried out first with guinea pig uterine muscle obtained from an animal that had been sensitized to horse serum. Such muscle was found to react violently to small amounts of horse serum added to the bath and also to histamine. Furthermore, uterine strips, obtained from patients who had not received serum previously, were tested with horse serum in order to determine whether or not spontaneous contraction would occur following the addition of this serum. No such reaction was obtained with the uterine strips of three control patients following the addition of horse serum, whereas the addition of pituitrin or of histamine gave definite reaction, indicating that the muscle strips were responsive to proper stimuli.

Although several patients were given the antistreptococcic serum, in only one of these was it possible to obtain the ideal conditions satisfactory for our experiment—namely, the securing of a muscle strip at a time when skin sensitization was evident and when circulating precipitins and reagins were present in the blood stream. In the latter patient, the addition of horse serum to the bath in which the uterine strip was immersed produced absolutely no contraction, whereas pituitrin and histamine produced definite responses. Although this experiment seems to show that artificial sensitization of human uterine muscle cannot be induced, definite substantiation of this fact could only be obtained by similar results in other cases. Unfortunately, ideal subjects or conditions for this type of experiment are not easily obtained and may require a long period of waiting—for this reason, the present summary is offered merely as a preliminary report until further evidence can be supplied. It is our intention to carry out the same type of experiment both on uterine muscle strips and, if possible, on strips of muscle obtained from the intestines, in order to vary the organ from which the smooth muscle is obtained.

THE JEWISH HOSPITAL OF BROOKLYN
DIVISION OF APPLIED IMMUNOLOGY

BROOKLYN, N. Y., NOVEMBER, 22, 1937

Studies in Anaphylaxis in the Rhesus Monkey. MURRAY ALBERT, M.D. (Abstract.)

The present experiments were undertaken an in attempt to study the reported phenomenon of anaphylaxis in the *Rhesus* monkey, as described by previous investigators.

Passive sensitization was attempted in 5 monkeys by single intravenous injections of from 28 to 40 c.c. of rabbit anti-egg serum of high precipitin titer (1:20,000—ring method). The testing dose, 10 c.c. of 50 per cent or 100 per cent egg white, was administered intravenously, after an interval of from four to twenty-four hours. No evidence of sensitivity was noted in any animal. Active sensitization was attempted by multiple intravenous injections of from 10 to 20 c.c. of 50 per cent or 100 per cent egg white at intervals varying from one to four weeks. Each animal received from two to six injections. Fatal reactions occurred in 3 out of 13 animals. These occurred in instances where the interval between injections was greater than two weeks and where 100 per cent egg white was used as the shocking agent. Precipitins could not be demonstrated in these animals. The fatal reactions consisted of increased respiratory and cardiac rates, convulsions, a period of apnea followed by gasping, and death within from three to seven minutes, with no evidence of obstruction to respiration. A similar death occurred in one instance in a normal animal in which 100 per cent egg white was injected for the first time.

The intestinal strips of 5 animals in whom active or passive sensitization was attempted were tested for sensitivity by the Schultz-Dale technique. No specific contractions of the intestinal strip were elicited by the addition of the antigen to the Dale bath.

These results of the experiments raise considerable doubt as to the specific immunologic nature of the so-called anaphylactic reactions reported by previous workers.

The Relation of the Dust Reaction to Other Inhalant Reactions. MURRAY ALBERT, M.D., KATHERINE BOWMAN, A.B., AND MATTHEW WALZER, M.D. (Abstract.)

Intracutaneous tests with inhalants were performed on 54 atopic and 4 nonatopic adults. The sera of these patients were then studied by the

Prausnitz-Küstner technique for the presence of reagins for dust and for the other inhalant allergens to which they manifested positive reactions on direct testing.

The one-plus reactions to dust manifested by the 4 nonatopic cases could not be transferred by the Prausnitz-Küstner technique.

The findings on the 54 atopic cases were as follows: 22 of them manifested a one-plus reaction to dust, 36.4 per cent of which could be transferred; 24 a two-plus reaction, 66.6 per cent of which could be transferred; and 8 gave a three- or four-plus reaction, 100 per cent of which were transferred.

In every instance where dust reagins were found, reagins were also present to some other dust-producing inhalant, such as wool, feathers, goat, silk, cottonseed, flaxseed, kapok, pyrethrum, horse, cat, dog, or rabbit dander.

An analysis of the records of 97 cases in private practice, consisting mostly of children suffering with all types of allergic illnesses in which tests had been performed by the indirect method revealed similar findings. In 72 of these cases, dust reagins were found; in 15, reagins for dust were absent. In every instance a positive dust transfer was associated with one or more transfers to other inhalants.

Reagins for pollens and for foods were found to occur independently of reagins for dust.

Studies in Contact Dermatitis. III. Active Sensitization Induced With Krameria. MAX GROLNICK, M.D. (Abstract.)

Experiments in active sensitization of the skin of human beings were carried out with Krameria or Rhatany, a plant substance not heretofore reported as a cause of contact dermatitis. Krameria is the dried root of the plants *K. triandra* or *K. argentea* and is used primarily as an astringent of the mucous membrane of the intestinal tract. The fluid extract used in these experiments contained 1 gram of Krameria in 1 c.c. of the extract, the vehicle being 60 per cent alcohol and 40 per cent water.

Patch tests with the Krameria were done on 100 subjects. Seven positive reactions appeared within the first week and were interpreted as evidence of preexisting sensitivity to Krameria, or to a closely related substance.

An attempt was made to induce sensitivity in 37 subjects who were negative on the initial test with Krameria. A patch test was applied to the unbroken skin of the arm for forty-eight hours. If no reaction resulted, a twenty-four-hour application was made at another site after a lapse of two weeks and at weekly intervals thereafter until a positive reaction was obtained. Thirty-two subjects (86.3 per cent) were suc-

cessfully sensitized by from one to eight applications. In 5 subjects (13.7 per cent) sensitization could not be induced with from seven to ten applications.

An analysis of the findings obtained on 10 subjects, who became sensitive with a single application of the excitant, revealed that a reaction first appeared in these subjects from the tenth to the twenty-first day after the patch was applied.

In 12 subjects the development of the hypersensitive state marked by the appearance of a positive reaction at the last site tested, was followed in from two to eleven days by an activation of the site of the preceding application which up to that point had remained unchanged. In three subjects, the appearance of sensitization was followed in from two to seven days by an activation of two of the previously negative test sites, in the reverse order of their application.

Sensitivity to Krameria is a generalized phenomenon. Experimental sensitization in human beings has been shown thus far to persist for at least seven months. In a patient who had become clinically sensitive to Krameria, a positive reaction to it was obtained after a lapse of four years.

Krameria appears to be an effective excitant of contact dermatitis in man and lends itself to experimental investigations in human beings because of the comparative ease with which it induces skin sensitization, the mild and localized reactions which it produces, and the relatively innocuous type of sensitivity it excites.

The Allergic Reaction in the Passively Sensitized Mucous Membranes of the Ileum and Colon in Humans. IRVING GRAY, M.D., MAX HARTEN, M.D., AND MATTHEW WALZER, M.D. (Abstract.)

The allergic reactions of the passively locally sensitized mucous membranes of the ileum and colon were studied on two individuals, one of whom had a colostomy, and the other an ileocolostomy.

The exposed mucous membranes of the ileum and colon were sensitized to peanut protein, about 2 inches from the skin edge of the openings, by the intramucosal injection of 0.05 c.c. of human serum containing reagin antibodies for peanut in high titer. A cutaneous site on the arm of each subject was also sensitized. From twenty-four to ninety-six hours after sensitization, the allergic reactions were induced at the sensitized mucous membrane sites by the administration of a peanut "milk," made by dissolving 10 gm. of raw ground peanuts in 30 c.c. of water. In three experiments this was given orally; in two, it was introduced through a catheter directly into the lumen of the bowel. In one instance the antigen was applied on an applicator directly to the sensitized area of the mucous membrane.

The allergic reactions which developed after several minutes at the sensitized mucous membrane sites were characterized by edema, hyperemia and increased mucous secretion. They appeared within five to eight minutes after administration of the test meal, and usually preceded the cutaneous reactions by several minutes. In one experiment the mere application to the mucous membrane of a cotton swab, holding about 0.1 c.c. of peanut "milk," was sufficient to induce allergic reactions at the sensitized cutaneous and mucous membrane sites. Examination of the mucous secretion produced by the allergic reaction failed to show the presence of eosinophiles.

The Absorption of Antigen From the Vagina and Cervix in Humans. MAXWELL ROSENZWEIG, M.D., AND MATTHEW WALZER, M.D. (Abstract.)

A direct method for studying the absorption of undigested protein from the human vagina and cervix was presented. The technique is similar to that employed for studying the absorption of unaltered protein from the gastrointestinal tract.

The subject was passively and locally sensitized on the flexor surface of the forearm by an intracutaneous injection of 0.05 c.c. of a 1:10 dilution of a serum containing reagins for peanut in high titer. The absorption test was performed twenty-four or forty-eight hours later. For this a peanut solution was used consisting of 5 gm. of raw ground peanuts in 15 c.c. of water.

In studying absorption from the vagina, the cervix was covered with a metal cap and a gauze sponge saturated with the peanut solution was inserted into the vagina. In studying cervical absorption, a cotton applicator was dipped in the peanut solution and gently inserted for a distance of one-quarter to one-half inch into the cervical canal, where it was left in place. Absorption of the peanut protein into the circulation was marked by the appearance at the sensitized site on the forearm of a reaction characterized by pruritus, erythema, and wheal formation.

Twenty-one patients were studied. Absorption from the cervix was found to be a normal phenomenon occurring in every case studied in from nine to twenty-five minutes. Absorption from the vagina was inconstant. It occurred in 6 out of 17 cases. The absorption time was from forty to one hundred twenty minutes.

REPORT OF THE JOINT COMMITTEE ON STANDARDS

Since the establishment in 1931 of this Committee by the Society for the Study of Asthma and Allied Conditions and the Association for the Study of Allergy, there have been submitted to the Committee a total of fifty-five applications from allergy clinics from the various parts of the country requesting the Committee's approval. Of these, forty were approved and fifteen were not approved. In arriving at approval, the criteria which continue to guide the Committee in its judgment of clinics are the following:

1. The clinic must be connected with a Class A Hospital with adequate facilities for hospitalizing and fully investigating its patients when necessary
2. Adequate medical personnel
3. Adequate clinic facilities
4. Adequate patient material
5. Adequate testing material
6. Adequate medical records

While the questionnaire, which must be filled out by clinics seeking recognition, inquires into research facilities and activities, it is stressed that such research activity is not considered a requirement for approval. Rejections have been, in practically every instance, due either to the fact that the clinic was not connected with a Class A Hospital or that the records submitted were poor.

In addition to approving new clinics the Committee feels that there ought to be some form of check-up, particularly on clinics already approved. The first step in this direction has been taken by sending a questionnaire to all approved clinics requesting information as to its present staff personnel, major clinic changes since the clinic was first approved, the yearly census of new and old cases seen in the clinic, and a list of publications made by the members of its staff.

The Committee further advises that there be made an inspection, both of clinics applying for approval, and at regular intervals of clinics that have been approved. Such a plan is to be submitted to the two Societies for action at their next meeting.

The Committee is prepared to consider applications for approval from any allergy clinic, irrespective of the clinic director's membership in either Society. Requests for approval should be directed to the Secretary. The next meeting of the Committee will be held on Saturday, December 3, 1938.

For the Society for the Study of Asthma and Allied Conditions:

J. Alexander Clarke, Jr., Philadelphia
Robert A. Cooke, New York
Leslie N. Gay, Baltimore

For the Association for the Study of Allergy:

Richard A. Kern, University Hospital, Philadelphia (Secretary)
George Piness, Los Angeles
Francis M. Rackemann, Boston

List of Hospitals With Allergy Clinics That Have Been Approved by the Joint Committee on Standards for the Study of Allergy and the Society for the Study of Asthma and Allied Conditions

ARIZONA

Phoenix

1. St. Luke's Home

CALIFORNIA

Los Angeles

2. Children's Hospital
3. Los Angeles County General Hospital
4. Santa Fe Coast Lines Hospital

DISTRICT OF COLUMBIA

Washington

5. Children's Hospital

INDIANA

Indianapolis

6. James Whitcomb Riley Hospital for Children

MARYLAND

Baltimore

7. Johns Hopkins Hospital

MASSACHUSETTS

Boston

8. Beth Israel Hospital
9. Massachusetts General Hospital

MINNESOTA

Rochester

10. Mayo Clinic

MISSOURI

St. Louis

11. Barnes Hospital
12. McMillan Hospital

NEW JERSEY

Montclair

13. Mountainside Hospital

NEW YORK

Brooklyn

14. Jewish Hospital

New York

15. Bellevue Hospital, Third Division
16. Bellevue Hospital, Children's Division
17. Beth Israel Hospital
18. French Hospital
19. Gouverneur Hospital
20. Lebanon Hospital
21. Midtown Hospital
22. New York Hospital
23. New York Eye and Ear Hospital
24. New York Post-Graduate Hospital, Dept. of Medicine
25. New York Post-Graduate Hospital, Dept. of Pediatrics
26. New York University Medical Clinic
27. Roosevelt Hospital
28. St. Luke's Hospital
29. Sydenham Hospital

Rochester

30. Rochester General Hospital
31. Strong Memorial Hospital

PENNSYLVANIA

Philadelphia

32. Children's Hospital
33. Graduate Hospital of the University of Pennsylvania
34. Hospital of the University of Pennsylvania
35. Jefferson Hospital
36. Mount Sinai Hospital
37. Pennsylvania Hospital
38. Philadelphia General Hospital
39. Temple University Hospital

Pittsburgh

40. University of Pittsburgh Hospital Falk Clinic

Annual Review

ALLERGY IN DERMATOLOGY: A CRITICAL REVIEW OF RECENT CONTRIBUTIONS*

MARION B. SULZBERGER, M.D., NEW YORK, N. Y., AND
JOSEPH GOODMAN, M.D., BOSTON, MASS.

ECZEMATOUS DERMATITIS OF ALLERGIC ORIGIN
CONTACT DERMATITIS

GENERAL discussions of eczema continue to be numerous,[1] indicating that there is even today no general agreement about the definition of the term "eczema." Before proceeding with a discussion of the recent literature, the authors wish to repeat what was stated in the first review of this series (1936). "Allergic eczema can today comprise only dermatitides possessing the clinical and histologic characteristics of eczema, and proved to be due to a specifically acquired state of hypersensitivity." Allergic eczema, therefore, is synonymous with contact dermatitis due to specific sensitization. There are other dermatoses which, on morphologic, clinical, and histologic grounds, must be included in the eczema group. However, many of these diseases are not yet proved to be of allergic nature and many are of unknown etiology. Others are dermatoses produced by the primary irritant substances, for the action of which no preceding sensitization is necessary. The skin reaction produced by the first application to the skin of croton oil, a primary irritant, may therefore be called eczema, but it may not be termed allergic eczema or allergic contact dermatitis. In these reviews the terms "eczema" and "contact dermatitis" are used in reference to allergic diseases, unless otherwise noted.

Everyone interested in this problem will welcome the complete and definitive consideration of eczema in the new text of dermatology by the late Josef Jadassohn.[2] The concept of eczema which we have just outlined and which we have always sought to conserve is indeed Jadassohn's concept, the viewpoint which he developed during his many fruitful years of investigation of eczema and kindred diseases. The first chapter of his text (*Eczema Vulgare*) is, therefore, recommended most warmly to all who are interested in the problem of eczema.

Statistical studies of contact dermatitis continue to be important. Oller[3] presents the results of patch-test studies, on 150 individuals with

*From the Skin and Cancer Unit, New York Post-Graduate Medical School and Hospital of Columbia University, and the Department of Dermatology and Syphilology, Massachusetts General Hospital and Harvard Medical School.

26 common eczematogenous substances. (It is not made clear in the summary of this paper whether or not these individuals were suffering from skin diseases at or just before the time of testing.) Positive reactions to patch tests were found in 81 per cent of the patients; 80 per cent of the reactors showed polyvalent reactions. In many instances there was a correlation between the positive reactions and the occupation or previous contacts of the patient. Seven patients with tuberculosis of the skin showed no reactions.

This study emphasizes again the frequency of polyvalent hypersensitivity in patients with contact dermatitis. However, at least two substances in Oller's test group are, in the authors' experience, rarely proved (although often suspected) to be the cause of allergic contact dermatitis. These are soap and silver nitrate. Unfortunately, the complete list of test substances is not given in the German summary.

Rostenberg, Jr., and Sulzberger[4] have reported a statistical study of over 10,000 patch tests in approximately 1,000 patients with various dermatoses. Patients with contact dermatitis presented a far higher percentage of reactions than did patients with other dermatoses. The group of patients with atopic dermatitis showed decidedly fewer positive reactions to patch tests than the contact dermatitis group. For many interesting findings the reader must be referred to the original paper, which is too complex for summary here.

The main interest in this study, however, lies in the demonstration by the authors that one can establish statistically the relative probability that a given substance will produce eczematous hypersensitivity (sensitizing index) as well as the relative probability that a given individual will develop allergic contact-type hypersensitivity (sensitization index). Such studies will furnish a new approach to problems affecting the consumer as well as industry. It should be possible to discover by a relatively simple procedure which substances are potentially harmful —from the point of view of their sensitizing capacities—and which are not. It should enable industry to substitute scientific studies of chemicals, dyes, food, and cosmetic ingredients, etc.—as well as of the suitability of prospective employees—for the present haphazard, expensive, and often dangerous trial and error method.

Mayr[5] presents a follow-up study of 300 patients with contact dermatitis, treated between 1926 and 1933. In 1937, the previously demonstrated allergic hypersensitivity had disappeared in about one-half of the cases. Only 13 per cent of the male patients in this group had been forced to change their occupations because of their hypersensitivities.

That 50 per cent of patients with contact dermatitis lose their hypersensitivities seems an unusual finding. We have a definite impression that once established, contact hypersensitivity is in many cases quite likely to persist unchanged for years. Further studies of this question are urgently required.

Most important among studies of the pathogenesis of contact eczema as well as of the pathogenesis of other varieties of allergic hypersensitivity is the work of Landsteiner and his collaborators. The more recent of these studies[6] continue to support Landsteiner's hypothesis that the formation of conjugated antigens is an important and common step in the production of skin hypersensitivity as well as anaphylactic hypersensitivity to simple chemicals. In certain groups of compounds (the halogen- and nitro-substituted benzenes) it was found that the capacity to sensitize guinea pigs in general ran parallel to the alkalilability of the compounds and their capacity for combining with aniline.

Since there is a reasonable doubt that contact-type dermatitis in man can as yet be completely identified with the hypersensitivity demonstrated in guinea pigs and other animals by means of intracutaneous injection, or even by external application of simple chemicals, it is important that Landsteiner's hypothesis be tested on human skins. It is of considerable interest, therefore, that Sulzberger and Baer,[7] in a study of the sensitizing capacities of five different substituted benzenes, found that, for human beings also, skin sensitizing capacity and the capacity to produce allergy of eczematous type ran roughly parallel to the alkalilability and the capacity of the compounds to combine with aniline.

Nitti, Bovet, and Depierre[8] have followed Landsteiner's general method of investigation in studying the sensitization of guinea pigs with paraphenylenediamine and related compounds. They demonstrated that paraphenylenediamine sensitized guinea pigs regularly when injected subcutaneously. However, the pigs sensitized to paraphenylenediamine showed no evidence of sensitization to the ortho- or meta- isomers of this substance, nor did these isomers produce an allergic hypersensitivity to themselves or to paraphenylenediamine. After studying various related substituted benzenes, the authors concluded that the sensitizing properties of paraphenylenediamine depend (1) on the molecular structure of the substance, the para position being of particular importance, and (2) on the lability and capacity for reaction of the amino groups. Thus Landsteiner's hypothesis receives additional support from this study of paraphenylenediamine and related compounds.

Robert[9] has contributed an excellent and fundamental study of the allergic and the primary irritant aspects of skin reactions to staphylococcus toxin. In only one-fourth to one-third of the 170 individuals tested did he find a reciprocal relationship between the reactions to the patch and the intracutaneous test on the one hand, and the staphylococcus antitoxin titer of the blood on the other. After studying various fractions of the filtrate of the bouillon culture of staphylococci, Robert came to the conclusion that the positive reaction to patch test with this material is not produced regularly by the same fraction. The bouillon filtrate is a mixture of many fractions, some of which act as primary irritants and others of which act as allergens.

This work is important in that it demonstrates, first, the presence of a multiplicity of (a) allergenic and (b) toxic and irritant factors in the staphylococcus, and second, the importance of considering these various factors in the evaluation of reactions to patch tests to staphylococcal bacterial products. Robert's work is complementary to the work of W. Jadassohn and his collaborators (discussed in previous reviews) on the antigen analysis by means of the Schultz-Dale technique, of trichophytin, tuberculin, and other similar bacterial antigens (see p. 411 of this review).

Also of considerable theoretical importance are the studies of Strauss and Coca on poison ivy hypersensitivity in the rhesus monkey. Strauss[10] first demonstrated that the skin of the rhesus monkey becomes sensitized to poison ivy just as do human skins. Whereas the guinea pig and the rabbit show a primary irritant reaction to the first application of concentrated poison ivy extract, the rhesus monkey and the human skin show a reaction only after sensitization has been established. Strauss and Coca[11] then studied in the rhesus monkey the spread of eczematous hypersensitivity to poison ivy extract. A full thickness cuff of skin encircling the monkey's upper arm was turned down and covered with a sterile dressing. The interruption of the continuity of the skin created by this operation constituted a barrier to the spread of the eczematous hypersensitivity to poison ivy extract. Monkeys sensitized by application only to a skin site on the operated arm, below the cuff, were later found to be hypersensitive to poison ivy extract only below the cuff on the sensitized arm. Conversely, animals sensitized on the unoperated arm showed a generalized spread of the hypersensitivity to all skin areas, except those below the cuff on the operated arm.

This observation, although as yet unconfirmed, is of considerable interest, for it would indicate that eczematous hypersensitivity spreads through the skin itself and not by any other route. Moreover, the authors conclude that these observations are to be interpreted as showing that in all likelihood the spread of this form of hypersensitivity is through the epithelial cells and not through the cutis. Further study of the important questions raised by this experiment is urgently needed.

Kile and Pepple[12] have contributed an interesting study of poison ivy hypersensitivity in the guinea pig. Regardless of the diet at the time of experimental sensitization to poison ivy, guinea pigs which were deficient in vitamin C at the time of testing to poison ivy showed a lesser degree of hypersensitivity than control animals on a balanced diet. The subsequent administration of vitamin C to these animals led to the development of a degree of hypersensitivity equal to that produced in the control group. A group of guinea pigs on a diet high in vitamin C developed a degree of hypersensitivity comparable to the control group.

It is of interest to compare these observations with those of Sulzberger and Oser, discussed in last year's review, on the effect of cevitamic acid on the sensitization of guinea pigs to arsphenamine. (See also the work of Cormia discussed in the section on Drug Eruptions.) Large doses of cevitamic acid apparently inhibited the development in the guinea pig of the tuberculin-type of hypersensitivity to arsphenamine, but in Kile and Pepple's experiments had no effect on the development in the guinea pig of hypersensitivities to poison ivy extract painted on the skin. The most important contribution which Kile and Pepple make, is the observation that the reduced sensitivity to poison ivy of the animal kept on a diet inadequate in vitamin C is a reversible phenomenon (perhaps a form of cachectic anergy—as pointed out by Sulzberger and Oser); and that the usual degree of hypersensitivity can be restored by the administration of vitamin C, without a subsequent sensitizing exposure.

However, while new light is being thrown on nonspecific factors affecting sensitivity, the problem of immunization to contact allergens is apparently no nearer solution. Thus far, successful desensitization has been reported only with plant oils. Black and Coca have reported the prevention of poison ivy by the intramuscular injection of poison ivy extract (see last year's review). Caulfield, confirming older observations, reports the successful preventive treatment of dermatitis from the handling of tulip bulbs[13] and the prevention of poison ivy by the intramuscular injection of a potent poison ivy extract.[14] Brunsting,[15] on the other hand, reported poor results in the treatment of twenty-four patients with ragweed dermatitis, only 5 of the group having derived any benefit from intramuscular injections of the oil fraction of ragweed pollen. This discrepancy of results and its possible causes were discussed in the last annual review.

One of the most interesting reports of the year is that of Loveman[16] concerning skin and mucous membrane sensitivity to oil of anise. There was a positive reaction to patch test with oil of anise. The stomatitis was reproduced by the chewing of candy flavored with oil of anise, but no reaction resulted from the swallowing of capsules containing oil of anise.

Contact-type hypersensitivity of the mucous membranes is certainly quite rare, as evidenced by the common absence of mucous membrane lesions even in patients with severe and widespread contact dermatitis. However, Loveman's thorough study of his case firmly establishes the exceptional existence of this variety of hypersensitivity in the mucous membranes, as also demonstrated in the case of Herbert Ratner.

The study of Kammer and Callahan,[17] on the other hand, does not impress us as having established beyond doubt the nature of torch oil dermatitis. The relationship of fungous infection and contact hyper-

sensitivity (especially industrial) has been pointed out before and has been commented on in previous reviews. That it plays a part in torch oil dermatitis seems not unlikely. However, these studies leave one in doubt whether torch oil dermatitis is a reaction produced by primary irritants or whether it is a true allergic hypersensitivity.

The reviewers[18] have reported a series of patients with hypersensitivity to dyes contained in lipstick and other cosmetics and in clothing (dresses, socks, shoes). Further details of the dye hypersensitivity in the lipstick cases were communicated at last year's meeting of the American Dermatological Association.[19] In summary, the studies demonstrated that about one-half of the 14 lipstick-hypersensitive patients were sensitive to eosin (the indelible dye) and most of the remaining patients were hypersensitive to l-sulpho-β-naphthaleneazo-β-napthol. In addition, patch tests with 20 common lipstick dyes revealed a polyvalent sensitization in many of the patients. It is of practical importance to note that in one patient the hypersensitivity was limited to the lips; no reactions to patch tests could be produced on any other areas of the patient's skin. Her hypersensitivity was, however, demonstrated beyond doubt by the burning and swelling of the lips which occurred repeatedly within a few hours of the use of the lipstick. All of the patients in this series who cooperated adequately (with the exception of the patient just mentioned) were able to use lipstick specially prepared for them, using only dyes to which they had negative patch tests. In the patient with sensitivity confined to the lips, the testing had to be carried out by the tedious method of local application (lip tests).

Not noted in last year's review were the cases of lipstick hypersensitivity reported by Sézary and his collaborators[20, 21] and by Gougerot.[22] The last named observer reported that photosensitization was an important factor in the production of cheilitis (one case each caused by red wine, lipstick, and fresh figs—each producing the reaction only when combined with light). Our observations could not bring convincing proofs that photosensitization was an important factor in any of our cases of lipstick hypersensitivity. It must be admitted, however, that our studies have not been adequate to enable us to reach a definite decision on this point.

Shoe dermatitis is an important variety of contact-type dermatitis and is not uncommonly due to leather dyes. Not infrequently patients presenting itching eruptions from shoes are treated for fungous infections for long periods, the true nature of the dermatosis going unrecognized. In the above-mentioned communication[18] the reviewers have discussed the differential diagnosis of fungous infection of the feet and shoe dermatitis. In these investigations, which were primarily concerned with dyes which produce eczematous dermatitis, the significant observation was made that one of the *dye-sensitive patients suffered from chronic*

urticaria which could be shown in all likelihood to be due to a lipstick dye. It was pointed out that dyes, and particularly dye-protein conjugates, are known to be anaphylactogenic and urticariogenic. For this reason, commonly encountered dyed materials (e.g., in house dusts, carpets, drapes, furniture, clothing, etc.) may be potent, important, and as yet insufficiently considered factors in the production of asthma, vasomotor rhinitis, chronic urticaria and other diseases of this group.

Niles[23] has recently published the details of a case of shoe dermatitis.

Dermatitis from dyes in clothing is not uncommon in the reviewers' experience. We have been able to confirm Bonnevie and Genner's clinical description of such cases (see last year's review). Our patients, however, showed hypersensitivity not only to blue clothing, but also to clothing of all other colors. Valade[24] has reported an additional case of dress dermatitis.

Among the interesting reports of contact dermatitis may be mentioned those of Milian and Garnier[25] and of Sézary and Horowitz[26] concerning contact hypersensitivity to lanolin. Although such hypersensitivity has been described previously, this condition merits renewed attention since, as first shown by one of the reviewers and Morse, patients with a hypersensitivity to lanolin present extremely difficult therapeutic problems if their allergy is unrecognized. The reviewers recently observed a patient with a lanolin hypersensitivity and in addition, a hand eczema which had been treated for years without benefit. The use of topical remedies which did not contain lanolin led to a rapid cure of the eczema. In passing, it should be noted that the rare hypersensitivities to boric acid and to other normally harmless and soothing topical remedies present a similar problem and may also go unrecognized for years.

Of interest also is the report of O'Leary and his collaborators[27] concerning an urticarial and eczematous dermatitis occurring after injections of iodobismitol. Patch testing revealed that the eruption was produced by saligenin, a local anesthetic contained in the iodobismitol. The dermatologist must be always on the watch for drug eruptions produced by vehicles or adjuvants, both of which are often considered too unimportant to be mentioned on labels.

ATOPIC DERMATITIS

Hill's studies of infantile eczema continue to be of considerable interest. In his most recent paper[28] he studied again the reaction of the atopic and the normal baby to house dust and goose feathers. It will be recalled that Peck and Salomon[29] reported the finding in infantile eczema of positive patch tests to house dust and goose feathers. Hill obtained no positive reactions, using their technique in 25 cases. It was pointed out at that time that the babies in New York City, studied by Peck and Salomon, frequently slept on feather pillows, while the Boston babies,

tested by Hill, usually did not. Hill then tested 74 infantile eczema patients under one year of age by the scratch method and found only two reactions to dust and one to feathers. Tests by the intracutaneous method, however, performed subsequently on 44 patients, showed 31 positive reactions to dust and 25 to feathers. Control intracutaneous tests on normal infants were entirely negative. Hill concludes that his infants with eczema have quite commonly a specific hypersensitivity to dust and goose feathers.

In general Hill has found that the removal of the substance to which the infant with eczema is skin hypersensitive—as demonstrated by the wheal reaction to tests—will sometimes cure the eczema. He has found milk, eggs, and wheat to be especially important. Other allergens, he believes, should also be eliminated so far as possible.

However, while infants with eczema may respond favorably to dietary elimination, children or adults with atopic dermatitis present a much more difficult problem. In our material treatment of these patients by the elimination of specific allergens has for the most part been disappointing. Mulzer and Sörensen-Petersen[30] have reported the results of treating patients with atopic dermatitis with a sodium chloride-free diet. In 16 cases it was found that the addition of sodium chloride to the diet regularly produced flares of the dermatitis. In all, 68 cases were treated by the salt-free diet alone, topical remedies being limited to lanolin or petrolatum. Fifty-one cases were "practically cured," and 16 were definitely improved. In all cases the itching was lessened quite promptly. An objective improvement of the skin usually appeared for the most part only after four to six weeks. It was found that even after long periods of time the addition of sodium chloride to the diet resulted in recurrences of the eruption.

Certainly a nonspecific therapy for this disease would be most welcome. While the results reported by Mulzer and Sörensen-Petersen seem quite promising, we wish to make mention, on the other hand, of the extreme difficulty of carrying out a salt-free diet. Patients on such diets often develop a salt hunger so extreme that the carrying out of the diet in the home becomes impossible. Therefore, it would seem necessary to reserve a trial of the "salt-free" diet for those cases in which, because of the severity of the eruption, the patient is determined to cooperate with the physician to the fullest degree.

The study of the leucopenic index in patients with atopic dermatitis, reported by Rusten,[31] demonstrates again the difficulties of specific elimination in such patients. Ten per cent of the foods which gave compatible curves, 35 per cent of those which gave indeterminate curves, and 50 per cent of those which gave incompatible curves caused pruritus or increase of the eruption. Moreover, although foods produced symptoms in about 75 per cent of the patients studied, they were rarely the sole cause of the eruption.

This study again emphasizes the fact that adequate elimination of allergens in patients with atopic dermatitis is rarely possible. However, this does not mean that elimination should not be attempted. On the contrary, patients with this distressing disease may sometimes be greatly benefited by proper diet and by the elimination of inhalant and environmental allergens, although very few indeed are entirely or permanently relieved by such procedures alone. It has seemed to us in general, that the younger the patient the more efficacious the therapy by elimination of allergens; and that in infantile atopic dermatitis, change of diet and more particularly of environment, controls the eruption in about 50 per cent of all cases (see article by the reviewers, Ref. 34 in last year's review).

Rollin[32] and O'Leary and his collaborators[33] have reported additional cases of juvenile cataract associated with atopic dermatitis. The literature (see last year's review) and our own experience indicate that this complication is found only in patients with atopic dermatitis of considerable severity, with extreme and multiple hypersensitivities. Atopic cataract may nonetheless occur early in the course of the disease, even within the first year. The paucity of reported cases indicates that it is fortunately a relatively rare complication.

URTICARIA AND URTICARIAL REACTIONS TO SKIN TESTS

The lack of a suitable animal for the study of atopic diseases has been a bar to the experimental study of atopy. The report by Strauss[34] that the rhesus monkey will accept passive transference of local skin hypersensitivity from human reaginic sera indicates that adequate experimental study of atopy may be possible with this animal. Strauss[10] has also demonstrated that the rhesus monkey can be sensitized to poison ivy. It seems possible, therefore, that further studies in the rhesus monkey of allergy, both of the atopic and the contact variety, will become increasingly important.

Baldwin and Glaser[35] have studied the effect of specific desensitization on the skin and mucous membrane reactions of patients with hay fever. A comparison of the specific skin reaction before and after desensitization showed no definite trend. In more than half of their cases the skin reaction was definitely less after desensitization, but in none had it disappeared entirely. However, not all of the patients who were improved by treatment showed a diminished reaction to skin tests; and, on the other hand, a few patients who derived no benefit from treatment nevertheless showed a definitely diminished skin-test reaction.

The results of Baldwin and Glaser may be explained, at least in part, by a modification of the urticarial skin reaction by nonspecific influences. A demonstration that such modification is possible has been furnished by Albus.[36] In a patient with symptoms of pollen hypersensitivity, the intramuscular injection of 2 c.c. of omnadin (nonspecific protein) six or

seven hours before testing, resulted in a positive test to a pollen which had previously given negative tests. This experiment is an interesting demonstration of a fact well recognized by those practiced in skin testing—namely, that the size of the urticarial skin reaction is not necessarily or always a measure of the degree of hypersensitivity to the test substance. Albus' result is also another example of the dermatologically well-known synergic effects of unrelated substances seen in either antagonism of different antigens ("Konkurrenz der Antigene") or in the specific polyvalence or nonspecific increase of effect through addition of two or more antigens ("Potenzierung"). The not uncommon "nonspecific flareups" are examples of the latter.

It must be further borne in mind that not all wheal reactions are allergic. Reactions to such primarily urticariogenic agents as morphine, codeine, atropine, pilocarpine, histamine, and cholin surely cannot be considered as being of allergic nature. The fact that urticarial reactions to physical agents may not be allergic is well brought out in the studies by Blum and West[37] of a patient showing an urticarial response to blue and violet light. Careful studies were done of the wave lengths of the light which was effective in producing the urticarial reaction. As a result of these studies a carotenoid pigment was thought to be responsible for the photosensitization. The authors found that allergy in the usual sense of the word was not responsible for the production of the light sensitivity in their patient.

Unfortunately the year's studies have thrown practically no additional light on the baffling problems of acute and chronic urticaria.

DRUG ERUPTIONS

Inasmuch as specific desensitization to drugs has been thus far unsuccessful as a general practical measure, considerable interest has been aroused in the effect of nonspecific agents in decreasing or inhibiting sensitization, such as the work of Sulzberger and Oser referred to in last year's review. The studies of Cormia[38] on the influence of vitamin C on arsphenamine hypersensitivity in guinea pigs have confirmed the fact that animals deficient in vitamin C can be sensitized more easily than animals on a diet containing adequate amounts of vitamin C. After a period of high vitamin C intake (50 mg. daily for twenty-eight days), Cormia found it impossible to produce sensitization in the guinea pig. However, after twenty-eight days of vitamin C deficiency without any other dietary change, 7 of the 22 animals in the group developed reactions to intracutaneous tests with arsphenamine.

These experiments as well as those of Streitman and Weidman confirm and extend the original results of Sulzberger and Oser on the inhibitory effects of high doses of vitamin C in experimental sensitization of guinea pigs. However, the results of experiments on the guinea pig cannot be translated directly to man, especially in regard to contact-type derma-

titis. For, as we have pointed out, there is still room for doubt whether the guinea pig develops a type of hypersensitivity which corresponds entirely to human contact-type eczematous sensitization.

Moreover, the results of Kile and Pepple,[12] who studied the sensitizing effect of a typical contact allergen (poison ivy) on guinea pigs, must be considered in connection with Cormia's work (see discussion in section on Contact Dermatitis). It is apparent that there is a difference in the effect of vitamin C deficiency on poison ivy and on arsphenamine hypersensitivity in the guinea pig. On the one hand, the animal becomes sensitized, but the deficiency inhibits the response to a test with the specific allergen (poison ivy). On the other hand, the deficiency (if not too great—"cachectic anergy") seems to permit sensitization to occur, and remedying the deficiency abolishes the response to a test or a surplus of vitamin may even prevent sensitization from developing (arsphenamine). It is apparent that studies on the effect of vitamin C on contact allergy in man are urgently needed.

In connection with this work on vitamin C and sensitization to arsphenamine, it is of interest that Vauthey[39] has studied the effect of the addition of vitamin C to solutions of neoarsphenamine. Under certain conditions, 35 per cent of mice injected with neoarsphenamine die within eight hours. If instead of being used immediately the drug is used two or three hours after it is put into solution, the mortality rate is 70 per cent. The addition of vitamin C to the solution decreases the mortality of mice injected with the freshly mixed drug to about 20 per cent. If the drug mixed with vitamin C stands for two to three hours after being dissolved, the mortality does not rise!

While studies of the effect of vitamin C on arsphenamine sensitization are relatively recent, it has been common knowledge for some years that infants and children are decidedly less likely to become sensitized to the arsphenamines than are adults. Frei[40] recently demonstrated that the amount of neoarsphenamine which regularly sensitized the adult guinea pig failed to sensitize the guinea pig less than three weeks old. This effect he found to be due to a true lack of sensitization.

In discussing drug eruptions in last year's review we commented on the relative importance of sensitization effects as compared to toxicologic effects of drugs. In this connection we discussed the work of Mattice and Weisman[41] on the normal urinary excretion of arsenic and the effect of *sodium thiosulfate* on arsenic excretion. Ayres and Anderson[42] have recently published evidence to demonstrate again the efficacy of sodium thiosulfate in promoting the urinary excretion of arsenic. However, such studies must now be repeated on patients whose arsenic *intake* (exposure) is adequately controlled, as was done in the series of Mattice and Weisman. The problem of the effect of sodium thiosulfate on arsphenamine dermatitis must of course be considered entirely apart from the question of the effect of sodium thiosulfate on arsenic excre-

tion. In this regard our views have not changed. We still agree with the opinion expressed by Moore,[43] that from a clinical standpoint the drug is of no value in arsphenamine dermatitis.

The enormous increase in the use of *sulfanilamide* during the past few years has brought with it various drug eruptions. In addition to eczematous dermatitis these include morbilliform, scarlatiniform, and purpuric eruptions, and even generalized exfoliative dermatitis. Of particular interest are the reports of photosensitization by sulfanilamide (see for example Neuman and Sharlit[44]). It is apparent that this drug should be used with due caution and only when properly indicated.

Baker and Brunsting[45] have reported a papular and urticarial eruption caused by *sulfocyanates,* used in the treatment of hypertension. Sweitzer and Laymon[46] have described four cases of drug eruption caused by *barbiturates,* three of which were fatal. In each of these four cases the drug was continued six to ten days after the appearance of the eruption. Since even a single pharmacologically correct dose of a drug may result fatally in the hypersensitive individual, it is apparent that it is essential to discontinue all drugs immediately when patients are thought to be developing drug eruptions. A high index of suspicion for drug eruptions is of considerable importance. That this suspicion should be extended even to infants is indicated by the report of Hassmann[47] of a tuberous *bromide* eruption in an epileptic infant one month of age.

Gougerot and Degos[48] report multiple sensitivities to foods which developed immediately following an erythroderma caused by *gold* injections. The authors state that such sensitization is frequent after eruptions caused by gold, less frequent after arsphenamine dermatitis. The reviewers have not yet seen cases of this kind.

SKIN ALLERGY IN INFECTIOUS DISEASES

What may prove to be a most important advance in the treatment of infectious skin diseases is embodied in the recent development of staphylococcus toxoid. In general the reports of the past year tend to confirm the results of previous workers. Thus Gernez and Pannequin[49] have demonstrated that there is no relationship between the antitoxin titer of the blood and the skin reaction to the intradermal injection of staphylococcus toxoid. Nélis and van Mechelen,[50] Coulon,[51] Milian,[52] Wollman[53] and Bocage and his collaborators[54] agree in reporting excellent therapeutic results from the use of staphylococcus toxoid in furunculosis. In addition Milian, Wollman, and Bocage and associates, report good results in ecthyma, hidradenitis and sycosis vulgaris. However, the results in acne were poor. Our impressions coincide to some degree with those of Milian and Wollman, excepting that we have found staphylococcus toxoid of considerable benefit in some few cases of cystic acne and in the persistent chin acne of young adult woman, if given for

longer periods of time. In the usual juvenile acne our results are poor; and in many cases of furunculosis we have seen no definite benefit. On the other hand, our results in some cases of acne varioliformis have been definite, not to say striking. Moreover, we too have observed that the skin reaction to intracutaneous injection of both staphylococcus toxin and of toxoid is often independent of the blood titer in antitoxin (antidermonecrotic action in rabbits' skin); that is, patients with high blood titer may occasionally nevertheless have strong skin reactions, and patients with no demonstrable blood antitoxin sometimes have little or no skin reactions.

In fairness it must be pointed out that May[55] reports good results with staphylococcus toxoid only in acute infections, which may be expected to heal spontaneously. Also the comments of various English dermatologists to the paper of Panton[56] were on the whole unfavorable.

These discordant results indicate that sufficient work has not yet been done for a proper evaluation of staphylococcus toxoid in the treatment of skin diseases. In the reports cited above the strength of the toxoid used is not stated clearly, so that one cannot adequately compare the treatment of those who report success with the treatment of those who report poor results. In particular, further studies of the relationship of the blood antitoxin titer to the clinical response are urgently needed.

Jahnel[57] has presented an interesting report on the course of experimental syphilis in various animals, especially in certain hibernating rodents. The animals for the most part develop symptomless infections when inoculated with *Spirochaeta pallida*. However, the organisms disappear during hibernation. Jahnel discusses the factors which may be responsible for this naturally occurring cure (reduction of body temperature, reduction of basal metabolism) and points out the part played by similar natural immune processes in human syphilis.

The recent work of Turner[58] on the preservation of *Spirochaeta pallida* by rapid freezing at low temperatures may furnish an explanation of the findings of Jahnel. Spirochetes frozen rapidly and kept at a low temperature (–78° C.) preserve their virulence for months. On the other hand, spirochetes frozen equally rapidly but maintained at –10° C. or –20° C. remain infectious for less than two months. Previous to Turner's work it was known that *Spirochaeta pallida* lost its virulence after storage for a few weeks at icebox temperature. Therefore, it seems possible or even likely that the effects observed by Jahnel are a function of the body temperature, which during hibernation is just above 0° C.

Penacchi[59] studied superinfection with syphilis of patients with paresis. In 26 of 45 cases inoculated there was a successful superinfection. Twenty-three of these inoculations resulted in papular lesions which did not ulcerate. In 3 cases, however, there were primary and secondary lesions. The original of this report is not available to us. The

German summary does not state definitely the character of the so-called primary lesion, whether it was a typical chancre or whether it was a nodular lesion with the characteristics of tertiary syphilis. If typical chancres occurred in these 3 patients, then the opinions which we stated in last year's review must be modified in the light of this newer evidence; for we stated that "paretics have a skin allergy . . . to spirochetes, demonstrable by reinoculation, an allergy which never (sic) permits the production of chancres, but which results in either no lesions at all, or in lesions of tertiary and very rarely of secondary type."

TRICHOPHYTIN AND TUBERCULIN ALLERGY

In our last review we discussed studies of the immediate wheal reactions to trichophytin in patients with erysipelas-like lesions of the legs. Marcussen[60] has reported systematic studies of the immediate and the forty-eight-hour reaction to trichophytin in about 2,000 patients. He found immediate wheal reactions in 69 individuals. Usually when the immediate reaction was present, the forty-eight-hour reaction was absent (see the review on Allergy in Dermatology, J. ALLERGY **7**: 385, 1936). Furthermore the immediate wheal reaction was found for the most part in allergic (atopic?) individuals. Marcussen points out that certain of these wheal reactions may be caused by some part of the media (especially the peptone) on which the trichophyton fungus is grown. However, experiments with his own specially prepared fungous extract convinced him that a certain number of these immediate wheal reactions were caused by a hypersensitivity to a specific (protein?) fraction of the organism.

Lewis, Wise, and one of us (S.)[61] have reported a study of 6 patients with a delayed reaction to trichophytin. The response was absent at forty-eight hours, but was present when the patients were seen one week after the test was done. Each of these patients showed the usual forty-eight-hour type of reaction when he was retested. The authors point out that this change in reaction time may indicate only a change in the degree of sensitivity. It may, however, represent the production of sensitivity *de novo* by the injection of an extract (plus other factors), although in general it is most difficult to obtain such a sensitization.

W. Jadassohn, Schaaf, and their collaborators[62] have continued their important work on the analysis of composite antigens. They have demonstrated by means of the Schultz-Dale technique that extracts of various trichophyton fungi contain many different antigens, some peculiar to the variety, some common to one or more members of the group. Conversely, they found that human, bovine, and avian desiccated, tuberculin contain an antigen common to all and none peculiar to any one. One nonpathogenic acid-fast organism (*Bacillus phlei*) was found to contain no antigen found in desiccated tuberculin. Experiments with desiccated tuberculin and Ertuban (an aqueous extract of washed tubercle

bacilli) disclosed no common antigen. Comparison of human and bovine Ertuban disclosed the presence of a common antigen and also *antigens peculiar to each extract.*

The finding of a common antigen in human and bovine Ertuban and the demonstration that each contains in addition at least one specific antigen peculiar to itself, indicates again that tuberculin, like trichophytin, is a mixture of many antigens. Even old tuberculin certainly does not contain all of the antigens of the tubercle bacillus.

In the light of these findings, the comparison of the Mantoux test and the intradermal test with purified protein derivative (P. P. D.) of the tubercle bacillus is of considerable interest. Low and Cory[63] found that the first standard test dose of purified protein derivative (0.00002 mg.) produced reactions in approximately the same number of individuals as did old tuberculin 1:10,000 (0.01 mg.). The second test dose of purified protein derivative (0.005 mg.) revealed seven times as many reactors as old tuberculin 1:1,000 (0.1 mg.), and twice as many as old tuberculin 1:100 (1.0 mg.). In a group of 732 children of low economic status the authors found 80 reactors. Of these, 55 reacted to both old tuberculin and purified protein derivative, but 25 reacted to the latter alone. In 6 individuals of this group of 25 there was x-ray evidence of pulmonary tuberculosis.

This study indicates that the reactions to purified protein derivative are not exactly equivalent to reactions to old tuberculin, and that each may contain different antigens. Further comparisons of this type are therefore necessary in order to determine the exact status of the newly introduced variables. Such studies may prove that the use of purified protein derivative is of some value in routine skin testing designed to uncover individuals with active or latent pulmonary or glandular tuberculosis. As yet no study has been reported of the reaction of patients with tuberculosis of the skin to purified protein derivative.

A study by Hayes and his collaborators[64] of the reaction of patients with active pulmonary tuberculosis to purified protein derivative confirmed in general the results of previous studies with old tuberculin. On the whole the level of sensitivity was low, and the course of the skin reaction could not be correlated with the course of the pulmonary infection. These authors were unable to establish any correlation between the skin sensitivity to purified protein derivative on the one hand and the patients' temperature or seasonal influences on the other. Appel and his collaborators[65] also studied this problem using Seibert's ultrafiltrate, trichloracetic acid precipitate tuberculin (T. P. T.), T. P. T. in tablet form and old tuberculin. Their results also led them to conclude that in pulmonary tuberculosis there is no relationship between the skin reaction to tuberculin and the prognosis.

Howe[66] has studied the daily variations in the reaction to purified protein derivative, using test doses of 0.00001 to 0.001 mg. and record-

ing the result as the product of the size of the reaction and the negative logarithm of the dilution. Four subjects with pulmonary tuberculosis and two normal subjects showed marked daily fluctuations, varying from 100 per cent to 50 per cent maximal reactions to the doses used. Howe analyzed the curves in relation to daily readings of diastolic blood pressure and daily meteorological readings. He found 77 per cent to 100 per cent correlation in the 6 subjects. Periods of peripheral vasodilatation, characterized by a falling diastolic blood pressure, are accompanied by an increased reaction to tuberculin. Conversely, periods of peripheral vasoconstriction, characterized by a rising diastolic blood pressure, are accompanied by a decreased reaction to tuberculin. The author concludes that "the mechanism of these variations in the tuberculin reaction appears to be one of periodic changes in vascular and tissue permeability, affecting the extent of local exudation in response to a definite dose of tuberculin. The increased permeability may also result in an actually increased sensitivity of the cells to tuberculin."

An important aspect of repeated tuberculin tests has not been discussed by Howe, namely, the possible desensitizing effect of one or more tuberculin tests. The reviewers' experience with desensitization procedures for patients with tuberculosis of the skin makes them feel that the degree of sensitivity to the tuberculin test can in many instances be definitely decreased by intracutaneous injections, although it cannot be made to disappear completely. In still other instances the intracutaneous injection of tuberculin either produces no change or results in an increased sensitivity. It has seemed to us that the induced changes in tuberculin sensitivity were usually transitory, many of the patients tending to return to their original degree of skin sensitivity after variable periods of time.

To previous papers on tuberculin patch tests[67] Wolff and Hurwitz have added a new study[68] of the comparison of the tuberculin patch test to the Mantoux test. In 1,075 cases there was 98.2 per cent agreement. The patch test was positive in every case of active tuberculosis. The authors feel that this method of testing merits a thorough clinical trial.

The results of Wolff and Hurwitz do not agree with results previously reported by Puente,[69] who also compared the patch test and the Mantoux test in 446 patients. He found strongly positive reactions in most cases of tuberculosis of the skin, but among 58 cases of pulmonary tuberculosis there were only two reactions to the patch test. Otherwise, however, the two tests corresponded fairly well. There were no positive patch tests in patients with negative Mantoux tests.

Great care must be exercised in interpreting the skin reactions to tuberculin patch tests as well as to patch tests with other substances. The description of the variety of positive tests given both by Wolff and

Hurwitz and by Puente make the reviewers suspect that in both instances at least two kinds of reaction were being observed. On the one hand, Wolff and Hurwitz describe vesicular reactions, and both reports make mention of bullous reactions. This variety of reaction is characteristic of, although not limited to contact-type dermatitis. On the other hand, both papers report reactions which consist of yellowish discoloration or a small yellowish membrane. Such reactions are most often (although not exclusively) caused by primary irritant substances. The histopathologic studies of this patch test reaction contained in Puente's report revealed some findings which were consistent with contact dermatitis and some which were more consistent with primary irritant reactions. Moreover, no note is made in Puente's report of the finding of any of the histologic characteristics of a tuberculoid reaction although previous observers have reported these characteristic histologic findings. The clinical and histologic evidence, therefore, does not indicate the exact nature of this patch test response.

The reviewers believe that continued studies of the patch test and other tests with tuberculin will disclose a basic difference in the mechanism of the various responses—the site and type of sensitization being different for the eczematous patch test response as contrasted with the immediate wheal reaction and also with the forty-eight hour usual reaction to Mantoux tests. In fact it may be that the different allergens in the tubercle bacillus produce these different types of response in differently sensitized individuals. We have already discussed the evidence that a staphylococcus bouillon filtrate contains contact allergens and primary irritant substances which probably differ from those which produce the forty-eight-hour response to the intradermal injection of staphylococcus vaccine. We have also presented the evidence that desiccated tuberculin and Ertuban do not contain a common antigen but each contains an antigen peculiar to itself. Cavalli[70] has studied the exotuberculin of Finzi, which he found to produce reactions on intracutaneous injection much like those to old tuberculin. However, a comparison of the patch test with this new tuberculin and the intracutaneous injection of the same substance showed that the patch test was for the most part negative, even though the intradermal test was strongly positive. This result suggests that the positive intradermal reaction and the positive patch test may be due to different allergens, the fraction producing the positive patch test being absent from or in very low concentration in the exotuberculin of Finzi.* In the face of such findings and of many other studies in the older literature which point in the same direction, it is difficult for us to interpret the results of Wolff and Hurwitz without further extensive study. Until the mechanism of the patch test reaction to tuberculin is better understood and until the various new

*Details of the preparation of this substance are not given in the German summary. The original is not available to us.

tuberculins such as purified protein derivative have been more extensively compared with old tuberculin, reliance should certainly be placed only on the quantitative graded Mantoux test with old tuberculin. It is our opinion that for some time to come it will be advisable to check each new form of test and each new preparation (both as yet represent unknowns) against a simultaneous intracutaneous old tuberculin test.

Rapoport[71] has reported studies of the effect of repeated injection of horse serum (5 to 6 intraperitoneal injections into the rabbit at five- to six-day intervals) on the development and localization of tuberculosis of the skin. The tubercle bacilli were injected intradermally or intravenously with the last injection of horse serum. The intradermal injection resulted in a chronic tuberculous ulcer which corresponded with the usual picture of a first infection. The intravenous injection of tubercle bacilli, however, produced a tuberculosis of the skin which, according to the author, corresponded to the picture of lupus vulgaris in the human being. The author concludes that these experiments demonstrate that a specific allergy to the tubercle bacillus is not necessary for the development of lupus vulgaris, but that a nonspecific allergy is sufficient.

This conclusion is certainly contrary to all the evidence produced by studies of the tuberculin sensitivity of patients with lupus vulgaris, for this type of tuberculosis of the skin is almost invariably accompanied by a high degree of tuberculin sensitivity. The difference in the reaction of the rabbits injected intravenously as compared to those injected intradermally is not an adequate basis for drawing conclusions about lupus vulgaris in man.

Bonnevie and With[72] report a patient who presented the clinical picture of sarcoid of Boeck. Clinically there were typical skin lesions and typical changes in the lungs and in the bones. The histology of the skin lesions was typical of Boeck's sarcoid. The Pirquet test was negative. After fourteen years, tuberculosis of a vertebra and of the urogenital tract developed, the lung involvement became more marked, and the skin and bone lesions regressed or disappeared. At the same time the Pirquet test became positive and remained so for three years. The clinical course and the behavior of the tuberculin test in this case certainly once again suggest a relationship between Boeck's sarcoid and tuberculous infection.

ALLERGIC MANIFESTATIONS OF THE SKIN WITH PARTICULAR REFERENCE TO THEIR SIGNIFICANCE IN GENERAL IMMUNOLOGY

In a previous review, the theoretical as well as the possible practical importance of sensitization to autochthonous substances was discussed. The possibility was pointed out that many obscure dermatologic diseases (as well as diseases important to the internist, the allergist, and the endocrinologist) may eventually be found to originate in some such sen-

sitization. We previously discussed the studies of Tuft,[73] Simon,[74] and Simon and Ryder[75] on sensitivity to insulin and pituitary extracts. Two further reports of such cases have appeared during the past year, neither of which, however, was as complete as the studies to which we have previously referred. Pendleton, Ball, and Rhode[76] report two obstetric patients who presented the picture of profound shock accompanied by urticaria, starting within one-half hour after the administration of pituitrin. The rarity of this type of sensitization is demonstrated by the fact that these two patients were the only ones among 15,000 obstetric patients to present this syndrome. Kocher[77] has also added a case of reaction to pituitary extract, in this instance administered for the relief of the pain of herpes zoster. The symptoms were somewhat the same as those in the cases just cited, excepting that the onset occurred three days after the conclusion of repeated small injections. The use of the word "anaphylaxis" in connection with this case is unfortunate.

Reports of such cases lead one to suspect that sensitivity to other organ derivatives must exist. In this connection the study of Criep[78] ranks with those of Tuft and of Simon and Ryder in importance. This report concerns a patient with pernicious anemia who presented evidence of atopic sensitivity, since he had hay fever and also gastrointestinal upsets and urticaria from various foods. After tolerating injections of liver extract without reaction for over a year, the patient suddenly developed asthma and urticaria of about twelve hours' duration after an injection of the usual variety of liver extract in the usual amount. Following the next injection one week later, the same symptoms occurred. The injections were discontinued. Five months later, however, liver extract was once more tolerated.

A study of this patient shortly after the onset of the sensitivity revealed a positive immediate wheal reaction to intradermal test with the liver extract with which he had been treated; and positive urticarial reactions to tests with extracts of hog, beef, sheep, and chicken liver. The patient's serum contained reagins to all of these liver extracts, as evidenced by positive passive transference. At the same time the intradermal test to serum and muscle extracts prepared from these same animals was entirely negative in result. A repetition of these tests three months later revealed the same skin reactions. Ten months after the onset of the hypersensitivity, there were still skin reactions to all of the livers except to that from the sheep, and to two commercial extracts prepared from hog liver. However, at that time the reactions to skin tests were less intense and the passive transference tests were negative. Precipitins to liver extract were present at the time of the first tests and at three months, but they had disappeared at the tenth month.

Criep's conclusion that this patient manifested a hypersensitivity to an organ-specific substance (a substance perhaps common even to all

mammalian and avian livers) and not to a species-specific substance is eminently justified by the results of his studies. Whether the hypersensitivity was directed toward the antianemic principle of liver was impossible to determine, since that substance has not been isolated in pure form. It seems probable in this case that the allergen was a "protein" present in all the livers tested. Thus to the organ-specific hypersensitivities to pituitary and to the islands of Langerhans of the pancreas, as well as to brain, lens, kidney, etc., we now add organ-specific hypersensitivity to liver. Studies of other organ-specific allergies will be of great value in emphasizing the importance of sensitization to autochthonous substances. In addition, allergists and dermatologists should in the future be on the alert for possible hypersensitivities to vitamins B_1 and C, both now available in pure form for injection.

One of the most important publications of the year is the monograph by Boström,[79] which is concerned with an in vitro method of studying allergic reactions. Using the Warburg respiration chamber, Boström studied the respiration and the anaerobic glycolysis of pieces of tissue removed both from animals and from human beings. He observed the change in these metabolic processes which occurred after the addition to the tissue of the specific allergen to which the donor was hypersensitive. Isolated slices of skin, liver, and kidney of normal guinea pigs showed no change when sera or purified proteins were added to them in the respiration chamber. However, when the tissue was removed from an animal with a specific allergic hypersensitivity, the addition to the tissue of the specific allergen resulted in a characteristic change in the respiration and anaerobic glycolysis. This reaction was called the "tissue metabolism reaction." The addition of large doses of the antigen resulted in a decrease or a cessation of the metabolic processes measured, moderate doses gave first a decrease and then an increase, small doses an increase only, and still smaller doses no reaction at all. The specific reaction was demonstrated in tissues removed both from actively and from passively sensitized guinea pigs.

Boström believes that the *tissue metabolism reaction* is an anaphylactic phenomenon, first, because the results derived from its use agreed in general with the usual tests for anaphylactic hypersensitivity (anaphylactic shock, Schultz-Dale experiment, passive transference, and precipitin tests); second, because a positive metabolism reaction could be produced only with the same substance used for sensitization (or with an immunologically related substance); and third, because in vitro desensitization of the tissue to the specific allergen could be demonstrated.

Attempts to reproduce the tissue metabolism reaction with histamine were unsuccessful, since large doses produced a drop in anerobic glycolysis, and small doses produced no change at all. In no instance did

histamine produce a rise in the metabolic reaction. The use of the proper concentration of the specific allergen, on the other hand, always produced a rise in anaerobic glycolysis.

These findings are an important addition the rapidly accumulating evidence that reactions of allergic hypersensitivity and response to histamine are not identical; and that allergic reactions of various types are not necessarily all due to liberation or production of histamine.

Of great interest to the dermatologist and to the allergists are the results of studies of the tissue metabolism reaction of skin from patients with various dermatoses. Skin slices removed from five patients with erythema induratum showed a reaction to the addition of tuberculin which was in every way similar to that found in tissue from sensitized experimental animals. Material from three patients with erythema nodosum (all Mantoux positive) gave a positive metabolism reaction on the addition of tuberculin. Tissue from a fourth patient, who was Mantoux negative, gave a negative metabolism reaction. In two instances, tissue removed from patients with sarcoid skin lesions and a positive tuberculin test showed positive reactions. In seven other cases, all negative to tuberculin, the tissue metabolism reaction showed either a peculiar delayed reaction or a negative result. With material removed from 24 patients with syphilis of various sorts (secondary, latent, tertiary of the skin, central nervous system syphilis, and congenital syphilis) the in vivo luetin test corresponded quite closely to the in vitro test, excepting in secondary syphilis. In this condition, which is almost invariably accompanied by a weak or negative reaction to skin test with luetin, the tissue metabolism reaction was strongly positive. In a few instances in which normal and involved skin could be compared, it was found that tissue removed from the secondary lesion showed greater in vitro sensitivity to luetin than nearby normal skin. In two cases of lymphopathia venerea (lymphogranuloma inguinale, lymphogranuloma venereum) the reaction to the addition of Frei antigen was positive once and negative once. Seven cases of fungous infection were tested with trichophytin, with positive results in 6. A positive reaction was also found in material from 6 of a group of 7 patients with noninfectious skin allergy (urticaria, atopic dermatitis, drug eruptions, and contact dermatitis).

The respiration and anaerobic glycolysis studied in these experiments is a function of epithelial cells. Sections of representative specimens of skin used in performing these experiments showed that the material studied consisted almost entirely of epithelium. Furthermore, the cutis itself did not show these reactions. Of great importance, therefore, is the demonstration that the epithelium plays an important role in many varieties of allergic phenomena. Perhaps not only in contact dermatitis, but also in the allergies which are characterized both by the immediate wheal reaction and by the forty-eight-hour tuberculin type of reaction, the epithelium may play some part. Since the wheal reaction and the

tuberculin type of reaction are primarily vascular, these studies indicate an intimate interrelation between the epithelium and the vascular system of the skin.

Perhaps the greatest importance of these experiments lies in the fact that here, for the first time, a technique has been developed which permits a quantitative in vitro study of many varieties of allergic phenomena. Also with this technique it is possible for the first time to study the sensitivities of certain viscera (liver, kidney, etc.) as well as the sensitivities of the skin and mucous membranes. If this work can be confirmed, it may mark a turning point in the study not only of dermatologic allergy but of allergy in general.

A few miscellaneous studies remain to be mentioned. Haxthausen[80] has studied the Forssmann antibody titer of the blood of patients with skin and venereal diseases. No diseases were found which were accompanied by changes as marked as those found in infectious mononucleosis. However, pityriasis rosea and atopic infantile eczema were accompanied by a high average titer. On the other hand, there was no increase in the titer of patients with the seborrheic type of infantile eczema.

The reviewers must admit to great difficulty in distinguishing between atopic and seborrheic infantile eczema by clinical appearance. Any procedure which can supplement skin testing and the effect of elimination in making this distinction will be most welcome. The difficulty of differentiating these two forms of infantile eczema has been well brought out by Hill.[28]

Of considerable importance to the allergist and to the dermatologist is the influence of whealing on the rate of absorption of injected substances, recently studied by Abramson and Engel.[81] These authors found that dyes and blood pigment are absorbed much less rapidly from normal skin than from an area in which a wheal is formed by the injection of histamine or of ragweed pollen extract (in the ragweed sensitive individual). This study has important bearing on desensitization procedures, in which it is probably important to have a relatively slow absorption of allergen. For this reason, whenever possible, whealing should be avoided in such procedures.

Among the more general papers published during the past year we wish to mention that of the well-known dermatologist and allergist, Urbach,[82] on the treatment of allergic diseases. Although the methods of Urbach are not widely used in the United States, his work is worthy of careful consideration by every one interested in allergy and dermatology.

The reviewers must again make mention of the difficulty of the task of selecting the subject matter for this annual summary of progress in dermatologic allergy. Important contributions are unavoidably overlooked, so great is the mass of material which is published each year. We have attempted to emphasize the salient features of the year's progress,

the most important of which are (1) the studies of the basic features of contact-type dermatitis, and its relationship to other forms of allergy, (2) the continuation of work on the analysis of complex antigens, and (3) the studies of the tissue metabolism reaction. Taken as a whole, the year has been marked by noteworthy progress, particularly in these three subjects.

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Selected Abstracts

Asthma and Hay Fever

UNDER THE DIRECTION OF SAMUEL M. FEINBERG, M.D., CHICAGO

Treatment of Hay Fever With Alum-Precipitated Pollen Extract. Sledge, R. F.: U. S. Nav. M. Bull. **36**: 18, 1938.

At the suggestion of Harrison in 1934 Sledge treated a number of hay fever patients with alum-precipitated pollen extract. The latter was prepared to have a final concentration of 0.25 per cent of alum in each dilution. The largest volume given was 0.3 c.c. and the largest dose constituted 6,000 pollen units. Usually 10 or 12 doses were given, the largest number being 18. Sledge mentions that he has treated 96 cases with only two severe constitutional reactions. However, in the 40 cases which he tabulates in detail, more than 25 per cent are noted as having had systemic reactions. The 40 tabulated cases are composed of equal numbers of grass and ragweed sensitive individuals. The author asserts that this extract has given him better results than the nonalum-precipitated pollen extract, that constitutional reactions were reduced in frequency and severity, and that the number of treatments necessary to control symptoms were reduced in some cases.

Hypertonic Dextrose Solutions in the Treatment of Bronchial Asthma. Stoesser, A. V., and Cook, M. M.: Journal-Lancet **58**: 12, 1938.

The use of intravenous hypertonic dextrose solution in the treatment of intractable asthma in children is reported by the authors. They employed a 25 per cent solution and gave 150 to 200 c.c. In some instances they obtained better results by eliminating the sodium chloride from the solution. In two instances hypertonic sucrose injections caused aggravation of the asthmatic symptoms. Stoesser and Cook believe that the effect of dextrose injection is not due to its hypertonic action. They think that more likely the activity resides in the specific action of dextrose. As evidence for the latter view they remind us that one of the physiologic results of adrenalin is an increase in the blood glucose.

Molds as a Cause of Allergy. Harris, L. H.: Ohio State M. J. **34**: 158, 1938.

Harris discusses the general problem of mold allergy and reports 12 cases. Although some of these have other types of sensitivity in addition, most of them are apparently proved cases of clinical respiratory allergy to fungi. The symptoms of the majority of these patients were much aggravated during the summer months, although they did not coincide entirely with the pollen seasons. Some of the clinical observations previously recorded are re-emphasized by Harris. Among these are the tendency for the nasal and bronchial symptoms to persist as late as November and December, the early age of onset of symptoms, and the frequent history of intolerance or positive skin tests to yeast or mushroom. Of the 12 patients 11 reacted to alternaria, 5 to yeast, and 9 to aspergilli. One reacted to aspergilli only. Of 10 patients who were treated with mold extracts, 8 obtained relief amounting to 75 per cent or more.

Allergy to Liver Extract. Criep, L. H.: J. A. M. A. **110**: 506, 1938.

Criep describes the case of a patient who developed allergic symptoms to liver following its repeated injection for pernicious anemia. While this is by no means the first instance of such allergy described, this case has been more thoroughly investigated than the majority of those previously reported. The patient was a 41-year-old man who had had some clinical allergy, particularly ragweed hay fever. After a series of injections of liver extract for pernicious anemia, two severe attacks of asthma and urticaria occurred after the last treatments. The patient was able to tolerate autolyzed liver by mouth, and at the end of six months he was able to resume the liver injections without the production of constitutional reactions. Several interesting observations were made. By intradermal testing, positive reactions to commercial liver extracts were obtained. Similar, but not as strong, reactions were obtained with protein extracts (Coca's method) of the liver of beef, sheep, chicken, and hog. The sera and extracts of muscle protein of these animals gave negative reactions. Passive transfer reactions were obtained to the commercial and prepared liver extracts but not to the serum or muscle preparations. At the end of three months the direct and indirect reactions were still present. Ten months after the initial systemic reaction, the commercial liver extracts were showing weaker reactions, none occurring in dilutions higher than 1:100. The Coca extracts of livers of beef, chicken, and hog gave slight reactions in 1:10 concentrations, and the sheep preparation gave a negative reaction at this time. The passive transfer tests were negative. This is, then, an instance of allergy of the organ specific type.

A Study of Blood Magnesium in Allergic Individuals. Braden, A. H., and Braden, A. H., Jr.: South. M. J. **31**: 85, 1938.

Because of the physiologic antagonism between magnesium and calcium, the authors thought that a study of the blood magnesium in allergic individuals might throw some light on the mechanism of allergy. The blood magnesium in 30 allergic patients ranged from 2.35 mg. to 2.80 mg. and averaged 2.60 mg. per 100 c.c. This compares favorably with the average of 2.62 mg. established on control subjects by these authors. The presence or absence of an allergic attack had no influence on these figures.

Dermatology

UNDER THE DIRECTION OF MARION B. SULZBERGER, M.D., NEW YORK CITY, AND JOSEPH GOODMAN, M.D., BOSTON

Allergic Phenomena Produced by Certain Aromatic Amines. Nitti, F., Bovet, D., and Depierre, F.: Compt. rend. Soc. de biol. **124**: 1164, 1937.

Guinea pigs were sensitized with paraphenylenediamine (or related compounds) by the intracutaneous injection of 0.1 c.c. of a 1 per cent solution in water, daily for ten days. Tests were done twenty days after the completion of the sensitizing injections. Animals sensitized with paraphenylenediamine regularly showed an allergic reaction to this substance on retest, but showed no hypersensitivity to the ortho and meta isomers. These isomers, moreover, did not produce an allergic hypersensitivity to themselves or to paraphenylenediamine. Monoacetyl-paraphenylenediamine did not produce hypersensitivity to itself, but in a few instances did produce a hypersensitivity to paraphenylenediamine. Paraphenylsulfamid, para-aminotoluol and para-aminophenol did not sensitize the animals; 2-methoxy-1, 4-diaminobenzol and 2-methyl-1, 4-diaminobenzol were regularly strong sensitizers; 1, 2, 4-triamino-

benzol sensitizes not only to itself, but also in lesser degree to paraphenylenediamine. The addition of a nitro or carboxyl group in the 2 position to 1, 4 diaminobenzol results in compounds which do not sensitize.

The authors conclude from these findings that the sensitizing properties of paraphenylenediamine depend on the one hand on its molecular structure (the para position being particularly important) and on the other hand on the lability and capacity for reaction of the amino groups. The addition of the NH_2, CH_3, or OCH_3 radicals does not affect the sensitizing capacity. The addition of the COOH or NO_2 groups definitely decreases sensitizing capacity.

Animal Experiments on the Effect of Salvarsan on Nurslings. Frei, W.: Acta dermat.-venereol. **18:** 396, 1937.

Dermatologists have long known that infants rarely present eruptions from the arsphenamines. The author, in comparing the reaction of young and adult guinea pigs to neoarsphenamine, found that guinea pigs less than three weeks old were not made hypersensitive to neoarsphenamine by the injection of an amount of the drug sufficient to sensitize adult guinea pigs with regularity. Of a group of animals three weeks old, only a few became sensitized. Animals five weeks old were sensitized like adults. Three female animals, sensitized during pregnancy, produced non-sensitive offspring. These three females were hypersensitive to neoarsphenamine just before delivery, were not hypersensitive for a short time after delivery, but again became hypersensitive spontaneously a few weeks later.

Further Investigations of Poison-Ivy Hypersensitiveness in Guinea Pigs. Kile, R. L., and Pepple, A. W.: J. Invest. Dermat. **1:** 59, 1938.

Guinea pigs on a diet which was markedly deficient in vitamin C were sensitized to poison ivy by the application of 50 per cent poison ivy "oil" daily for four or five days to a manually depilated area of the abdomen. These pigs were less sensitive to subsequent testing with 5 per cent poison ivy oil than were controls with an adequate intake of vitamin C. However, after the vitamin C deficiency of these pigs was remedied, they were found to be as sensitive as the control group. On the other hand, a group of guinea pigs fed a diet which was relatively high in vitamin C were no more sensitive to 5 per cent poison ivy "oil" than was a control group. The authors have demonstrated for the first time that the lack of response of a group on a low vitamin C intake is due to a temporary, reversible effect and is not due to a true inhibition of sensitization.

The authors also found that irritation with a strong salicylic acid resorcin ointment painted with the 50 per cent poison ivy "oil" decreased the degree of sensitivity obtained. They also found that the offspring of female guinea pigs sensitized before or during pregnancy were not hypersensitive to poison ivy "oil."

Stomatitis Venenata. Report of a Case of Sensitivity of the Mucous Membranes and the Skin to Oil of Anise. Loveman, A. B.: Arch. Dermat. & Syph. **37:** 70, 1938.

The author reports a case of stomatitis and dermatitis of the left palm which was produced by contact with a dental plate—the stomatitis by wearing the plate and the dermatitis by holding the plate in the left hand while it was being cleaned. Careful patch testing revealed a hypersensitivity to oil of anise, an ingredient of the cream used for cleaning the plate. There was also a positive patch test to anethol (the chief constituent of oil of anise) and to oil of fennel (which also contains

anethol). The stomatitis was reproduced by chewing candy flavored with oil of anise. No reaction was produced by the taking of oil of anise in capsules, swallowed without being chewed. For eighteen months after discontinuing the use of the denture cream, the patient remained free from stomatitis and dermatitis.

Studies of Eczema. V. An Investigation of the Effect of Staphylococcus Toxin on the Skin. Robert, P.: Arch. f. Dermat. u. Syph. **175**: 530, 1937.

The author demonstrated in previous communications that it was possible to produce with filtrates of various bacterial and fungous organisms clinically, and in some instances histologically, typical positive patch test reactions. In an attempt to determine whether these patch test reactions are due to primary irritation or to allergic sensitization, the author investigated on 170 individuals the relationship between the patch test reaction to the filtrate of a staphylococcus culture, the reaction to the intracutaneous injection of staphylococcus toxin, and the staphylococcus antitoxin titer of the blood of the individuals tested. Toxin was heated to 100° C. for two hours. Certain individuals showed reactions to both the unheated filtrate and the heated preparation. Others showed reactions only to the unheated material. Furthermore, the reactions to heated toxin and to formolized staphylococcus toxoid did not correspond. In only one-fourth to one-third of the individuals was there a reciprocal relationship between the antitoxin titer of the blood and the patch or intracutaneous test. The protein fraction of the toxin, salted out with ammonium sulfate, showed neither positive patch nor positive intracutaneous tests.

On the other hand, the water soluble fraction recovered by precipitation with alcohol, gave positive reactions by both methods. The conclusion which Robert reaches is that the reactions noted are caused by various fractions of the bouillon filtrate which behave differently when subjected to heat, formalin, etc. The reactions are produced by a group of substances, some of which are primary irritants and others of which are allergens.

L'anatoxine staphylococcique en dermatologie. Milian, G.: Rev. franç. dermat. **13**: 67, 1937.

The author reports his results from the use of toxoid in various skin diseases. The toxoid was given in doses of from 0.5 to 3.0 c.c., at intervals of one week. (Strength of toxoid in dermonecrotic units not given—Eds.) Frequently two injections were found to be sufficient. Good results were obtained in furunculosis, hidradenitis, ecthyma, and sycosis vulgaris. The results were poor in patients with acne. Local and general reactions to injections were common.

Immunology

UNDER THE DIRECTION OF MATTHEW WALZER, M.D., BROOKLYN

Age and Antibody Production. III. Quantitative Studies on the Precipitin-Reaction Antisera Produced in Young and Adult Rabbits. Baumgartner, L.: J. Immunol. **33**: 477, 1937.

The precipitin reactions of rabbits, sensitized with horse serum or ovalbumin, were studied by the optimal proportions technique of Dean and Webb and the neutralization method of Culbertson. Quantitative chemical analyses, according to the technique of Heidelberger, were made of the precipitates made with young and adult rabbit antisera.

The author reports that precipitating sera, produced in young rabbits, tend to combine with specific antigen in different proportions than do antisera produced

in adult rabbits. Sera from young rabbits neutralized smaller amounts of crystalline ovalbumin than did sera of adult rabbits, even when both sera were of apparently identical titer. The amount of precipitin for crystalline ovalbumin formed on immunizing young rabbits is markedly less than that formed by adult rabbits.

The Vaccination of Guinea Pigs Against Tuberculosis With Dead Tubercle Bacilli Enveloped in a Covering of Vaseline. Saenz, A.: Compt. rend. Soc. de biol. **128**: 495, 1937.

The writer confirms previous reports that the vaccination of guinea pigs with dead tubercle bacilli, in a dry form or in saline, affords only very slight protection to reinfection with a virulent strain, administered after an interval of five weeks. However, if the same dose of dead tubercle bacilli is given in vaseline, and the animals are reinfected with the same virulent strain, only regional lymph gland infection is found in sixty to seventy-five days after reinfection. At this time the control animals show infection of the internal organs. The control animals die at the end of five months, whereas the animals injected with vaseline-coated tubercle bacilli live for eight months.

A New Tuberculin Patch Test. Vollmer, H., and Goldberger, S. W.: Am. J. Dis. Child. **54**: 1019, 1937.

The authors describe a new tuberculin patch test, recommended because of its simplicity.

Thin filter paper, saturated with tuberculin or bouillon control material, is suspended in a dust-free room and allowed to dry. Small squares of filter paper are placed on adhesive patches and applied directly to the skin. The moisture of the skin is sufficient to liquefy the tuberculin and cause the cutaneous reaction. A positive reaction manifests itself as a sharply defined indurated reddened square area with lichenoid follicular elevations of the skin.

Using this procedure, the writers report that the patch test conformed with the von Pirquet test in 187 (89.5 per cent) of 209 tuberculous children tested. Of the 22 cases where the two procedures did not produce the same results, 15 showed a positive patch test and a negative von Pirquet. On the basis of these findings, the authors conclude that the test is reliable for diagnostic purposes.

The Effect of Anaphylactic Shock on the Course of Infectious Disease. Otto, R., and Schaefer, W.: Ztschr. f. Hyg. u. Infektionskr. **120**: 230, 1937.

The writers studied the effect of anaphylactic shock on the course of infectious disease.

Guinea pigs, sensitized with horse serum, were infected with 1 c.c. of a 1:100 dilution of a twenty-four-hour bouillon culture of *Bact. enteritidis* Gaertner. The resulting infection produced an illness of from six to twelve days' duration with fairly constant autopsy findings. Forty-eight hours after onset of the infection some of the animals were shocked by the intracardiac or intraperitoneal injection of horse serum. On the basis of the findings in 195 sensitized and infected animals, the authors conclude that anaphylactic shock had no effect on the severity of the infection although the tempo of the infection was increased.

Anaphylaxis in the Isolated Heart. Wilcox, H. B., and Andrus, E. C.: J. Exper. Med. **67**: 169, 1938.

The hearts of young guinea pigs, which had been sensitized to horse serum, were isolated and perfused. Myographic and electrocardiographic tracings and measure-

ments of the rate of flow of the perfusate through the isolated heart were made under normal and experimental conditions.

The addition of from 0.01 to 0.1 c.c. of horse serum to the perfusate produced no unusual responses in the heart of the normal or of the desensitized guinea pig. The addition of from 0.002 to 0.005 c.c. of horse serum to the perfusing fluid entering the isolated heart of a sensitized guinea pig resulted, after one minute, in an acceleration of the heart beat. Electrocardiographic studies revealed a prolonged P-R interval and changes in the QRS complex. These changes lasted only from five to fifteen minutes. A striking reduction in the coronary flow was noted. Subsequent additions of horse serum resulted in less pronounced reaction or no reaction.

The injection of histamine into the perfusate of the normal or sensitized heart had an effect similar to that produced by the specific antigen on the sensitive heart. The reaction was shorter in duration than the specific one and did not produce desensitization.

The authors are of the opinion that a histamine-like substance is released during anaphylactic shock in the isolated and perfused heart of the sensitized guinea pig.

A Sheep Antibody Which Blocks the Prausnitz-Küstner Reaction. Cohen, M. B., and Nelson, T.: J. Immunol. **34**: 63, 1938.

The authors sought to determine whether sheep, receiving injections of pollen, would manufacture the same type of "blocking" antibody found by Cooke and his co-workers in the sera of human beings receiving pollen therapy for hay fever. The "blocking" antibody was reported as interfering with the Prausnitz-Küstner phenomenon in a characteristic manner.

Two sheep were injected, 12 and 13 times, with 50 to 80 c.c. and 20 to 40 c.c., respectively, of an aqueous extract of ragweed pollen, containing 1 mg. total nitrogen per cubic centimeter. The animals were bled at intervals, and the sera tested for the presence of the "blocking" substance. It was detected in both animals. Other workers, furnished with these sera, confirmed the authors' findings. No precipitin against pollen extract could be demonstrated in the sheep sera.

Pediatrics

UNDER THE DIRECTION OF WILLIAM C. DEAMER, M.D., NEW YORK

Round Table Discussion on Asthma and Hay Fever in Children. Ratner, B., Schmidt, W., and Stevens, F.: J. Pediat. **12**: 399, 1938.

Ratner feels that almost all adult allergy has its inception in childhood. He states that allergy in children is an acquired condition which appears within the first year in half the cases and before five years in 80 per cent of the cases. Three-fourths of the allergic children are food sensitive. While oral desensitization can usually be accomplished, the prevention of sensitivity is also possible and is accomplished by avoiding the introduction of undenatured foods in excessive amounts into the child's dietary. In particular, milk sensitivity is caused in the obstetric nursery by the use of complementary feedings, especially of improper forms of milk. Evaporated milk is the least likely to cause sensitivity.

Several of the participants stress the occurrence of unfavorable results from the use of 1:100 adrenalin, mentioning severe circulatory and gastric disturbances from its use.

Dr. Stevens mentions the use of autogenous and stock bacterial filtrates in the treatment of infectious asthma and the difficulties in using the skin test as a guide.

Transmission of Ragweed Pollen Antigen Through the Breast Milk. Sterling, A., and Fishman, A. E.: Arch. Pediat. **55**: 173, 1938.

A ragweed sensitive woman who was undergoing a course of desensitization with ragweed pollen antigen was delivered of a normal child. Treatment was suspended during the month after delivery and then resumed. One week after resuming treatment, at which time the third injection with 1:100 extract had been reached, the 5-weeks-old breast fed infant began to have paroxysmal attacks of sneezing and to cough and wheeze.

In an attempt to classify the cause of these symptoms, the mother's breast milk was used to test five individuals known to have positive ragweed skin tests. Presumably the tests were intradermal. Of the five, one individual gave a slightly positive reaction to two out of six samples of breast milk. The authors concluded that this was evidence for the presence of antigen in the breast milk. An attempt to demonstrate ragweed reagin in the same breast milk by passive transfer failed. As there were seven other instances of desensitization during pregnancy without noticeable ill effect, it is concluded that desensitization during pregnancy is harmless. The contrary evidence presumably presented by the above instance is ignored.

Concerning the Treatment of Bronchial Asthma in Childhood. Püschel, E.: Klin. Wchnschr. **17**: 313, 1938.

In the hope that a method of treating asthma in the adult which had been described by Dzsinick and Paul would prove successful, Püschel followed their technique on an asthmatic child of 5. The method called for intravenous magnophyllin to combat the increased affinity of the tissues for water, and increasing doses of histamine subcutaneously. The child received this treatment for three weeks in the hospital. Seventeen days after discharge she returned with another severe attack requiring hospitalization. The author concludes that methods apparently successful in adults may not be applicable to children, and that Vogt was right when he said that desensitization was not the entire answer to the problem of childhood asthma.

Treatment of Eczema in Breast Fed Infants. Sprenkel, V.: Arch. Pediat. **55**: 85, 1938.

The author confines this unique treatment to the eczema of exclusively breast fed infants who are not getting any other food. It consists in injecting the mother once a week for ten weeks with 5 c.c. of either normal saline, Ringer's solution, or distilled water. These may be given intravenously, subcutaneously, or intramuscularly, although distilled water is preferably given intravenously. Three-fourths of the cases of eczema treated in this way underwent permanent and successful cures, the majority within five weeks. Only 16 per cent of cases failed to respond at all. No explanation is offered as the basis of the treatment other than that it appears to be nonspecific. Its use was suggested by the observation that a majority of eczematous infants were born of mothers who suffered from toxemia of pregnancy, which responded to similar procedures.

Rhino-Ophthalmology

UNDER THE DIRECTION OF FRENCH HANSEL, M.D., ST. LOUIS

The Faucial Lesions of Purpura. Watson-Williams, E.: J. Laryng. & Otol. **53**: 181, 1938.

Three very interesting cases of purpura with faucial lesions were reported by Watson-Williams. In two instances the lesions followed the administration of

novarsenobenzol. In the first patient there was bleeding from both sides of the nasal septum. On the posterior wall of the pharynx there was a large purple bulla with bleeding edges. There was also bleeding from the tonsillar fossae. By endoscopic examination lesions were noted in the esophagus. A few purpuric spots were also noted on the extremities. Recovery followed discontinuation of the drug treatment.

The second patient showed violet-black staining of the mucosa of the anterior faucial pillars, the gum margins adjacent to the teeth in both jaws, and the inner surface of both cheeks opposite the molars. There were no cutaneous lesions in this case. The patient recovered after discontinuation of the injection of novarsenobenzol.

The third patient had purpuric lesions of the nose, mouth, and pharynx. She also had some purpuric skin lesions of the chin and neck. The purpura in this case followed the ingestion of "aspro" tablets. The faucial lesions were very extensive. The patient completely recovered.

Recurrent Paralysis of the Larynx Following Injection of Tetanus Antitoxin. Report of a Case. Neffson, A.: Arch. Otolaryng. **27**: 201, 1938.

A very interesting case of recurrent paralysis of the larynx following the injection of tetanus antitoxin was reported by Neffson. The patient was a male, aged 19 years, who, after the injection of 1,500 units of tetanus antitoxin developed first an urticaria, later generalized muscular and articular pains, and then suddenly a hoarseness. Examination of the larynx showed a complete paralysis of the left vocal cord and diminution of sensation on the left side of the larynx. The left pupil was slightly larger than the right. There was also an equivocal Babinski sign on the left, and questionable paresis of the right side of the face of central origin. The laryngeal paralysis improved gradually and disappeared completely three months after the onset.

Lysozyme of Nasal Mucus: Method of Preparation and Preliminary Report on Its Effect on Growth and Virulence of Common Pathogens of Paranasal Sinuses. Daly, S.: Arch. Otolaryng. **27**: 189, 1938.

Daly has shown that lysozyme is present in normal nasal mucus. He described a method of obtaining sterile preparations from nasal mucus. His experiments showed that nasal lysozyme in sufficient concentrations caused *Staphylococcus aureus, Streptococcus hemolyticus,* and pneumococcus III to grow in a granular manner. In one experiment pneumococcus III suffered some loss of virulence as a result of contact with nasal lysozyme. Although the results of these experiments on pneumococcus III were considered inconclusive, they seem worthy of note since they are in agreement with the findings of others.

Allergic Conjunctivitis Due to Fungi. Simon, F.: J. A. M. A. **110**: 440, 1928.

In a study of a case of conjunctivitis the following evidence indicated that the chief etiologic factor was an allergic reaction to the spores of air-borne fungi. Seasonal variations in the patient's symptoms coincided with seasonal variations in the atmospheric concentration of fungus spores. Changes in the patient's geographical location were accompanied by changes in his symptoms. Skin tests with fungus extracts were definitely positive. The clinical symptoms of conjunctivitis were reproduced by the application of fungus extract to the conjunctiva. The control test in a non-sensitive subject was negative. The

patient was exposed to fungus spores in that these were cultured in abundance in his home. Hyposensitization with fungus extracts was followed by relief of symptoms.

Miscellaneous

Hypersensitiveness to Digitalis. Brandt, R.: Wien. klin. Wchnschr. **50**: 1525, 1937.

A patient, a woman aged forty-six years, developed an eczematous lesion of the skin on several occasions. It was finally discovered that each time the skin manifestations were preceded by the oral ingestion of digitalis, taken for myocardial weakness. The drug was taken in the form of powdered digitalis leaves. On testing the patient at a later time with a different digitalis preparation, namely, Digilanid, in doses sufficient to produce a pharmacologic effect, no evidence of hypersensitization occurred. The extreme rarity of sensitivity to digitalis in this sense, rather to its pharmacologic effect, is pointed out.

Allergy to Liver Extract. Criep, L. H.: J. A. M. A. **110**: 506, 1938.

Only six previous instances of allergy to the injection of liver extract have been reported. The case here cited is that of a man forty-one years old with a history of hay fever. He received liver extract intravenously for pernicious anemia at intervals of ten days for a period of one year. Treatment was discontinued in April, 1936, and resumed the following month. Shortly after the third reinjection severe asthma and generalized urticaria developed. The same episodes followed the next injection. Autolyzed liver concentrate was substituted without untoward reaction. Six months later the hypersensitiveness to the original extract had disappeared.

A carefully controlled series of immunologic tests were performed and the following conclusions arrived at: the sensitivity is an acquired allergy and analogous to that to insulin and to solution of posterior pituitary. The sensitivity, moreover, is one to an organ and not to a biologic source. Positive skin tests and reagins are present for at least three months following the initial reaction. Loss of clinical sensitivity was coincident with the disappearance of reagins. Precipitins were demonstrable at the same time as reagins, but no anaphylactic bodies were demonstrable. This type of acquired allergy could not be produced experimentally.

Sensitization by Vitamin B_1 of Isolated Intestinal Strips of Rats and the Circulatory System of Cats to the Action of Acetylcholine. Agid, R., Beau Vallet, M., and Minz, B.: Compt. rend. Soc. d. biol. **127**: 982, 1937.

An intestinal strip from a rat suspended in a bath of oxygenated Ringer-Locke solution was tested with given amounts of acetylcholine and its contraction recorded. On adding vitamin B_1 in dilution of $1:10^{-6}$ and retesting with acetylcholine, a marked prolongation of contraction was observed.

The carotid blood pressure of a cat was taken. The administration of acetylcholine caused a characteristic fall, which was greatly enhanced with vitamin B_1.

The question arises whether anaphylactic and allergic disturbances, which are identical with acetylcholine effects, may be increased by vitamin B_1.